4-26-2018

Assessment of Mercury Content in Louisiana's Freshwater Fish and its Association to Se Concentrations

Alexander David Reyes-Avila
Louisiana State University and Agricultural and Mechanical College, alexanderadra@gmail.com

ASSESSMENT OF MERCURY CONTENT IN LOUISIANA'S FRESHWATER FISH AND ITS ASSOCIATION TO SELENIUM CONCENTRATIONS.

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Environmental Sciences

in

The Department of Environmental Sciences

by
Alexander David Reyes Avila
BSc., National University of Honduras, 2008
MBA., Centro American Technological University, 2012
August 2018

To all people in my country and around the world who have perished fighting for environmental justice and for the people working for a cleaner environment to be inherited by future generations.

----------Alexander Reyes-Avila

ACKNOWLEDGEMENTS

I really want to thank the Fulbright Laspau-administered program and Louisiana State University for supporting my education in the Graduate School. Thank you to the Department of Environmental Sciences for granting me admission to the master's program. I would also thank Dr. Edward Laws for orienting me through all these two years at the department, advising me to further my education successfully.

Special thanks to Thomas Blanchard, my chemist in the team, his guidance, advising and training were essential to the development of my thesis work. Very special thanks to Dr. Ronald DeLaune for all his support to my literature review and his continuous encouragement too.

Thanks also to Dr. Achim Hermann for his support testing and developing a method for Selenium analysis, his role was vital for completing my work on Se. Special thanks to Dr. Jim Wang and Dr. Vince Wilson for sharing tips during my thesis work. They were valuable members of my committee. Thanks to Dr. Slawo Lomnicki and Dean Lay for their collaboration with my lab work. I am also very grateful to Dr. Nicholas Ralston; whose papers inspired my idea and were central to complete my analysis.

Finally, very special thanks to Alex McClellan and Baoling Wang as well, for their continuous support during my lab work; their advice was vital piece to complete my research. Thanks to all faculty staff and classmates that supported my thesis work, specially to Charlotte G. St. Romain, her advising was essential for my success.

TABLE OF CONTENTS

ABSTRACT

Ample evidence has demonstrated the neurotoxic properties of organic Hg to humans. However, recent studies have proposed the protective effects of Se against organic Hg detected in marine fish. Louisiana's freshwater bodies are exploited by recreational anglers that enjoy fishing as recreational activity and food source. Thus, testing of Hg in Louisiana was resumed in 2017 to update the state advisories. However, before drawing conclusions based solely on organic Hg, it might be useful to see how much Se is present in freshwater fish.

The main objective of this study was to determine the Se:Hg molar ratio in Louisiana's freshwater fish; the ratios should be greater than 1.0 to expect Se's protective effects. Five waterbodies were surveyed (University lake, Calcasieu lake, Toledo Bend, Atchafalaya River, and Henderson lake). The last three are listed in the state advisory. The fish's fillet from species such as: Black drum, Catfish, Largemouth bass, Bluegill, Gizzard shad; were tested for total Hg via Direct Mercury Analyzer. Testing for Se used the same fish samples for determination via ICP-MS.

The results revealed Hg concentrations on Louisiana's fish were all under the 1 ppm EPA limit and LDEQ limit of 0.88 ppm (from 0.0063 to 0.67 ppm). However, Se concentrations were variable for different species and locations (from 0.024 to 0.886 ppm). Therefore, the calculated Se:Hg molar ratios were variable. Some ratios may suggest a relationship by species; like in Black drum and Catfish. Notwithstanding, large species (Bass) accumulate large amounts of Hg that exceed Se concentrations. That explained the low ratios for Se in Henderson lake's bass but, is not true for Atchafalaya's bass. Thus, fish from locations highly polluted with Hg apparently have Se:Hg molar ratios less than 1. There is no clear dominant variable (species or location) on the ratio determination.

In conclusion, the predicted variability of Se in freshwater fish by other scholars were observed in this study. Apparently, location and species are variables with unpredictable dominant roles. For proper evaluation of state advisory, both might be considered independently for any particular freshwater body.

1. INTRODUCTION

1.1. The Mercury Menace in Freshwater Bodies

Mercury (Hg) is a naturally occurring element, with a regular cycle in the environment. However, anthropogenic activities are now part of its cycle (EPA 2010). Consequently, Hg was mobilized and spread into the biosphere; increasing its presence in the environment after the industrial revolution until its peak in about 1970, when US and world production and consumption of Hg dropped dramatically (Laws 2018).

One of the inorganic species of mercury: Hg^{2+} is very commonly found in soil, sediments, and water. A small portion of this Hg (II) is transformed into methylmercury (MeHg) by bacteria. Once MeHg or organic Hg is produced, it can accumulate in fish tissue and undergo biological magnification from one trophic level to the next in aquatic food chains (EPA 2010).

The consumption of fish contaminated with organic Hg is of great concern from a public health standpoint. Hg, is a well-known neurotoxin that represents a threat for adults but, particularly women of childbearing age, and for a fetus. Therefore, in 2001 the United States Environmental Protection Agency (USEPA) started mapping Hg concentrations in fish collected from freshwater bodies all over the United States. By 2008, Hg had been detected in all states and territories. Thus, several states have issued advisories for fish caught in rivers, lakes, and even coastal waters, if the fish contain Hg at concentrations of 0.07 up to 1.0 ppm (EPA 2010).

1.2. The Louisiana Picture

Testing of Hg stopped during Governor Bobby Jindal's administration (2008-2016) because of cuts in the budget of the Department of Environmental Quality (Hardy 2017). Testing of Hg in Louisiana's fish was resumed in 2017, primarily motivated by the economic implications of resident's taste for seafood. Louisiana's coast possesses a large eager community of recreational anglers and a vastly productive fishery in the region of the Gulf of Mexico (Lincoln et al. 2011). A significant number of licensed recreational anglers consume the fish they catch from freshwater bodies in Louisiana (Lincoln et al. 2011). Health officials warned that Hg might be present in the fish caught by anglers. Indeed, relatively high concentrations of MeHg were detected in the hair of Louisiana anglers in 2006 (Lincoln et al. 2011). With new funding, it is expected that 50 bodies of water in Louisiana will have up-to-date advisories for consumption of fish (Hardy 2017).

1.3. The Se Argument

In Hawaii, state authorities have taken a somewhat different position with respect to fish consumption. A study by the University of Hawaii (U.H.) has suggested that most women of childbearing age can freely eat fish in Honolulu (Gutierrez 2012). The UH Medical school has revealed that the loss of benefits (Omega-3 polyunsaturated fatty acids and vitamins) that result from avoiding fish in the diet is more serious than the consequences of Hg exposure.

Table 1. Fish in human health

Source of essential polyunsaturated fatty acids such as arachidonate and eicosanoids
Reduced risk of heart attack by modulation of eicosanoids.
Reduced risk of bronchial asthma, psoriasis, diabetes, and autoimmune diseases
Fish’s fatty acid n-3 type can antagonize arachidonate conversion to harmful compounds
Fish’s n-6 fatty acids are essential vitamins materials (antioxidants)
Fish’s oil lowering of serum lipids
Fatty acids n-3 can lower brain damage caused by cerebral ischemia (stroke)
Fish meat decreases the amount of total fat and saturated fatty acids in the diet

Source: Fish and Human Health (Lands 1986).

Since 2010, the center for women and children and the UH Cancer Research Center have been conducting a study with a sample of 100 women and have monitored their consumption of fish during pregnancy. Researchers revealed that the element Selenium (Se), which is naturally present in fish, has benefits that outweigh the Hg toxicity. Apparently, Se is a protective element against Hg toxicity because it binds very strongly to Hg and effectively sequesters the mercury. According to Ralston (2010), Se can suppress the toxicity of MeHg because Hg's binding affinity for Se is roughly a million times its binding affinity for sulfur. Furthermore, Se is a nutraceutical with medical and health benefits. Disruptions of metabolic processes and diseases are related to inadequate amounts of Se in the diet (Ralston et al. 2010). Thus, Se in fish is beneficial both because it sequesters Hg and because it is a nutraceutical that replenish the depleted Se reserves by Hg toxicity. According to Ralston and Raymond (2010), Se is an essential trace element that is absolutely required for the physiological activity of 25-35 enzymes with vital functions in the brain and endocrine organs (2007). Marine fish and seafoods are good sources of dietary Se. Supplemental Se in the diet of a pregnant woman might protect the fetus from exposure to MeHg (Ralston 2010). A feeding study with rats showed after 5 weeks they were highly dependent on dietary Se to support growth and brain Se-enzyme synthesis. After depletion of their Se reserves by exposure to MeHg, impaired Se-enzymes activities were detected (Ralston 2016). However, Ralston (2010) has also warned that the concentrations of Se in freshwater fish are variable. For Se to adequately protect a consumer form Hg toxicity, the molar ratio of Hg to Se should be less than 1.0, and preferably much less than 1.0 (or Se to Hg ratio should be greater than 1.0). Knowledge of the Se:Hg ratio in freshwater fish should make it possible to make more informed assessments of the risk to recreational anglers who consume freshwater fish in Louisiana. If this ratio is much greater than 1.0, then fish advisories based on the amount of Hg in the fish may be unnecessary and in fact counterproductive from the standpoint of human health.

1.4. Project Objective

The main objective of this project was to determine the molar ratios of Se to Hg in freshwater fish taken from Louisiana freshwater systems. To accomplish this goal, two complementary objectives were carried out: First, I estimated the concentrations of total Hg and MeHg in freshwater fish samples caught in Louisiana waterbodies in the summer of 2017. Second, I determined the concentrations of Se in the same freshwater fish assayed for Hg. Next, I calculated the molar Se:Hg ratios in the fish and determined whether the ratios were higher or less than 1.0. Finally, I decided whether I felt there was a need to reassess current Louisiana's fish consumption advisories.

1.5. Research Design

1.5.1. Goal 1: Determination of Hg and MeHg

For this determination, I used the methodology of Carbonell et al. (2009) for MeHg with minor modifications. Total Hg and MeHg in the fish muscle tissue were determined via a direct mercury analyzer (DMA).

1.5.2. Goal 2: Determination of Se

Se, was measured using inductively coupled plasma mass spectrometry (ICP-MS).

1.5.3. Goal 3: Determination of the ratio of Se with respect to Total mercury.

I determined the molar Se:Hg ratios in the muscle tissue in accord with Kaneko et al. (2007).

1.5.4. Goal 4: Assess the implication of results in terms of fish consumption advisories

If the Se:Hg molar ratio in the fish were greater than 1.0, the current Hg advisories in Louisiana's freshwater fish should perhaps be reconsidered. However, if the ratio were less than 1.0, then a more thorough study is probably required to determine which fish species or waterbodies might be a threat.

2. MATERIALS AND METHODS

2.1. Terminology in Mercury Analysis

Total mercury means all forms of Hg. Inorganic mercury includes salts formed by mercuric mercury (Hg^{2+}) and mercurous mercury (Hg^{+}). Examples include mercuric chloride ($HgCl_2$) and mercurous chloride (HgCl), respectively. Organic mercury compounds are usually characterized by the formula HgR_2 or HgRX, where R is an aryl or alkyl group, and X is a halide or acetate. An example of an organic mercury compound is phenylmercury acetate, which has been used, inter alia, as a fungicide. The term methyl mercury (MeHg) refers to compounds of the form CH_3HgX (Laws 2018).

2.2. Sample Preparation for Methylmercury Analysis

For assessing MeHg, the methods proposed by Carbonell et al. were used with some minor modifications (2009). Essentially, this method uses microwave digestion to extract MeHg into toluene. The organic phase is then mixed with a solution of cysteine acetate, which selectively captures all MeHg. Finally, the toluene phase is removed from the cysteine phase, and the Hg is measured in the latter with the Direct mercury analyzer (Carbonell et al. 2009).

2.2.1. Digestion Process and Extraction of Methylmercury

To digest fish samples, I used a microwave-accelerated reaction system (model MARS-5®, CEM Corp., Matthews, NC). All the materials used for the analysis of MeHg and total Hg were rinsed with detergent and distilled water and then acid-washed with a 50:50 mixture of trace metal grade nitric acid and hydrochloric acid. The chemical reagents for digestion and extraction were sodium acetate (99.6%), L-cysteine hydrochloride monohydrate (99%), toluene (99.5%), and trace metal grade hydrochloric acid (30%) (Carbonell 2009). The steps for the sample preparation (including the modifications) are as follows:

a. Based on the EPA methods, a sample of 0.5 g wet weight (w/w) of fish tissue (from the filet) was placed in a Teflon vessel.

b. An aliquot of 750 µL of trace-metal-grade HCl (30%), 1000 µL of Milli-Q water (resistivity: 18.2 MΩ·cm), and 10 mL of toluene were added to the vessel

c. The Teflon vessels were tightly closed with the help of a CEM device designed to carefully seal the caps. Then, the samples were placed into the MARS-5® microwave oven.

d. The temperature of the microwave oven was programmed as follows: first, the temperature increased to 110°C in 10 min and then was held constant for 10 min. (Carbonell 2009)

e. After complete cool down of the vessels, 4 ml of the toluene phase was transferred to a 15-ml capped tube containing 2 ml of 1% cysteine acetate solution. (cysteine acetate (1%) was obtained by mixing L-cysteine hydrochloride (2%) and sodium acetate (2%) v/v) (Carbonell 2009).

f. Then, after 5 minutes of centrifugation at 3000 RPM, separation of two phases was achieved in the capped tubes.

g. The upper layer of toluene was removed with a Pasteur pipet, and the lower layer of cysteine with MeHg was ready for analysis.

h. Samples of approximately 100 mg of the cysteine phase were weighed and loaded in boats that were then introduced into the direct mercury analyzer DMA-80, Milestone SRL, where they were dried at 300°C and then thermally decomposed at 850°C. The Hg vapor was selectively trapped on a gold amalgamator. After the system was flushed with oxygen to remove any remaining gases or decomposition products, the amalgamator was rapidly heated, and the Hg vapor was released. Absorbance of the Hg vapor was measured at 253.7 nm (Carbonell 2009).

i. For a mercury standard, I used a standard reference material from the National Institute of Standards and Technology (NIST) with a precision of 1.1 ± 0.19 ppm.

j. For the blank, an empty boat was placed in the DMA-80.

k. Three replicates were analyzed for each sampled species.

2.3. Procedure for Total Mercury

For analysis of total Hg, the DMA-80 does not require any sample preparation (Milestone Srl 2013). The samples were cut with a scalpel (all materials were carefully rinsed with detergent and distilled water, and acid washed with a 50:50 mixture of trace metal grade nitric acid and hydrochloric acid), and their wet weights were then measured. Approximately 50 mg of fish tissue (from the filet) was directly loaded into boats that were introduced into the DMA-80 system for immediate analysis. Triplicate analyses were run for each species of fish tissue. Standard and blank were used as in MeHg analysis. All samples were immediately analyzed to prevent any potential absorption of Hg from the laboratory environment.

2.4. Sample Preparation for Se Analysis

Fish samples were analyzed for Se via ICP-MS. EPA method 3052 was used to remove all organic material from the fish tissue. The sample was digested in a microwave digestion system (SINEO® MDS-6G, Hanon Instruments, Jinan, China). It is essential that organics be completely removed because carbon enhances the Se signal in the ICP-MS. According to Nelms (2016), when samples contain carbon (dissolved CO_2, carbonates, or organics), the Se signal jumps significantly; for example: in a 2% (v/v) methanol (solvent), the Se signal increases by about a factor of three. The result is overestimates of Se concentrations (Nelms 2016). To completely remove the organics from the samples, hydrogen peroxide at 30 to 40% was used to oxidize the organic carbon in the matrix.

Because the Se concentrations in the fish tissue were low, it was necessary to design a suitable method for making concentrated samples so that Se could be detected via ICP-MS. The protocol I followed (including modifications to EPA method 3052) is summarized as follows:

1. Samples of 1.0 g wet weight from fish tissue were cut by scalpel (all materials were carefully soaked in Alconox® for 12 hours and then washed with nitric acid).

2. Each sample was placed in a Teflon vessel (previously cleaned and acid washed). Then, 8 mL of 67 to 70% trace metal grade nitric acid was added to each vessel, followed by the addition of 5 mL of 30-40% H_2O_2 the vessels were sealed, and the caps tightened.

3. Temperature and pressure regulators were connected to one vessel, and all the vessels were placed in a microwave digestion oven. The temperature in the oven was then varied as follows. The temperature was increased to 180 ± 5ºC in about 5 minutes and then held at 180 ± 5ºC for 10 minutes for the completion of reactions. Lastly, there was a period for cooling down the vessels.

4. Digested samples were placed individually in 15 ml Teflon beakers. The samples were placed in a hotplate and an inverted evaporation dish Pyrex® modify for ultra-pure air flow to allow almost complete evaporation of samples at temperatures up to 100-110ºC).

5. Additional aliquots of 5 mL of 30-40% H_2O_2 were added because of the presence of pale yellow organic material after the first evaporation. The Teflon beakers were then allowed to evaporate again, and more peroxide was added as required.

6. After complete evaporation and oxidation of organics in the sample, the remaining material was brought to approximately 7 mL with a standard solution of 1 ppm Germanium (Ge) and 1 ppm of Indium (In). According to EPA method 6020, it is required to use an appropriate internal standard such as In or Ge. They were added to make corrections for matrix effects, transportation effects, and thermal effects on the matrix. They help to keep the signal for the limit of detection in ICP-MS stable for all samples by working on these corrections.

7. Finally, the samples were placed in capped centrifugation tubes for ICP-MS analysis along with standards and reference material.

2.5. Preparation of Standards for Se Analysis

To prepare standards for the calibration curve, I used a 10-ppm/mL Se standard as the base solution for ICP-MS. This solution was in a matrix of nitric acid. My expectation was that the of Se concentrations in the fish tissue would be in the range 0.001 to 2 ppm. However, after an initial trial, it was apparent that the expected concentration range for selenium would be 0.1 to 1.0 ppm. The points for the standard calibration curve were therefore 0.1 ppm, 0.25 ppm, 0.5 ppm, 0.75 ppm, and 1.0 ppm. All five of these solutions were brought up to 25 mL with the same internal standard solution of 1 ppm Ge and 1 ppm In that I used for the fish samples. All these solutions were prepared gravimetrically and volumetrically as follows.

1. For the 0.1 ppm standard, 0.25 mL, or 0.254 g of the 10 ppm Se standard was brought up to a volume of 25 mL with the 1 ppm Ge-In solution in a volumetric flask.

2. For the 0.25 ppm standard, 0.625 mL, or 0.635 g of the 10 ppm Se standard was brought up to a volume of 25 mL with the 1 ppm Ge-In solution in a volumetric flask.

3. For the 0.5 ppm standard, 1.25mL, or 1.261 g of the 10 ppm Se standard was brought up to a volume of 25 mL with the 1 ppm Ge-In solution in a volumetric flask.

4. For the 0.75 ppm standard, 1.875 mL, or 1.882 g of the 10 ppm Se standard was brought up to a volume of 25 mL with the 1 ppm Ge-In solution in a volumetric flask.

5. For the 1.0 ppm standard, 2.5 mL, or 2.527 g of the 10 ppm Se standard was brought up to a volume of 25 mL with the 1 ppm Ge-In solution in a volumetric flask.

6. The blank for these standards and the sample was a volume of 25 mL of the 1 ppm Ge-In solution in a volumetric flask.

7. All the standards and samples were placed in centrifugation tubes to be assayed in the ICP-MS system.

2.6. Reference Material

To validate the result of the analysis, a Bovine Liver 1577b matrix was used as reference material. This was intended to prove that the methodology for the sample preparation completely oxidized all the organics and prevent carbon enhancement in the Se signal. A sample of 0.75 to 0.78g was weighed and digested exactly as the fish samples for Se analysis. The reference measurement reported a concentration of 0.419 ppm of Se. The certified value for bovine liver 1577b is 0.73 ± 0.06 µg/g or ppm. Then, the percentage of recovery was 66.7%.

2.7. Sampling Locations

Black Drum and Catfish sp. samples were purchased from a popular local market Tony's seafood in Baton Rouge, Louisiana. Largemouth Bass samples from Atchafalaya river and Henderson Lake were directly caught by Thomas Blanchard, Research Associate from the Department of Oceanography at LSU. The batch of samples coming from University lake at LSU included: Bluegill, Largemouth Bass, Gizzard Shad and Brown Bullhead Catfish. Dr. James Cowan Jr., professor of the Department of Oceanography at LSU identified each of these species through an organoleptic inspection assisted by an official Handbook about Louisiana Fisheries. All fish samples were stored in a freezer prior to analysis. All samples sites are exclusively located in Louisiana. Table 2.1 and Figure 2.1 show the sample locations and fish species.

Table 2. Locations for collected fish samples and species. Coordinates from Google maps®.

Species Samples	Scientific names	Number of samples	Location	Latitude and longitude
Black Drum	*Pogonias cromis*	10	A. Calcasieu Lake.	29°54'53.9"N, 93°17'19.4"W 29.91498, –93.28873
Catfish	Sp.	10	B. Toledo Bend.	31°35'28.4"N, 93°47'48.7"W 31.59121, –93.79687°
Largemouth Bass	*Micropterus salmoides*	10	C. Atchafalaya River.	30°20'25.1"N, 91°42'44.2"W 30.34031, –91.71228
Bluegill, Largemouth Bass, Brown BullHead Catfish, and Gizzard Shad.	*Eupomotis macrochirus Ameiurus nebulosus Dorosoma cepedianum*	17	D. University Lake LSU.	30°25'16.1"N, 91°10'10.0"W 30.42115, –91.16944
Largemouth Bass	*Micropterus salmoides*	10	E. Henderson Lake, Breaux Bridge, LA.	30°19'56.6"N, 91°45'02.5"W 30.33239, –91.75069

Note: Toledo Bend, Atchafalaya river, and Henderson Lake were the bodies of water under advisories by the LDEQ. Calcasieu Lake and University Lake at LSU were not under any advisory.

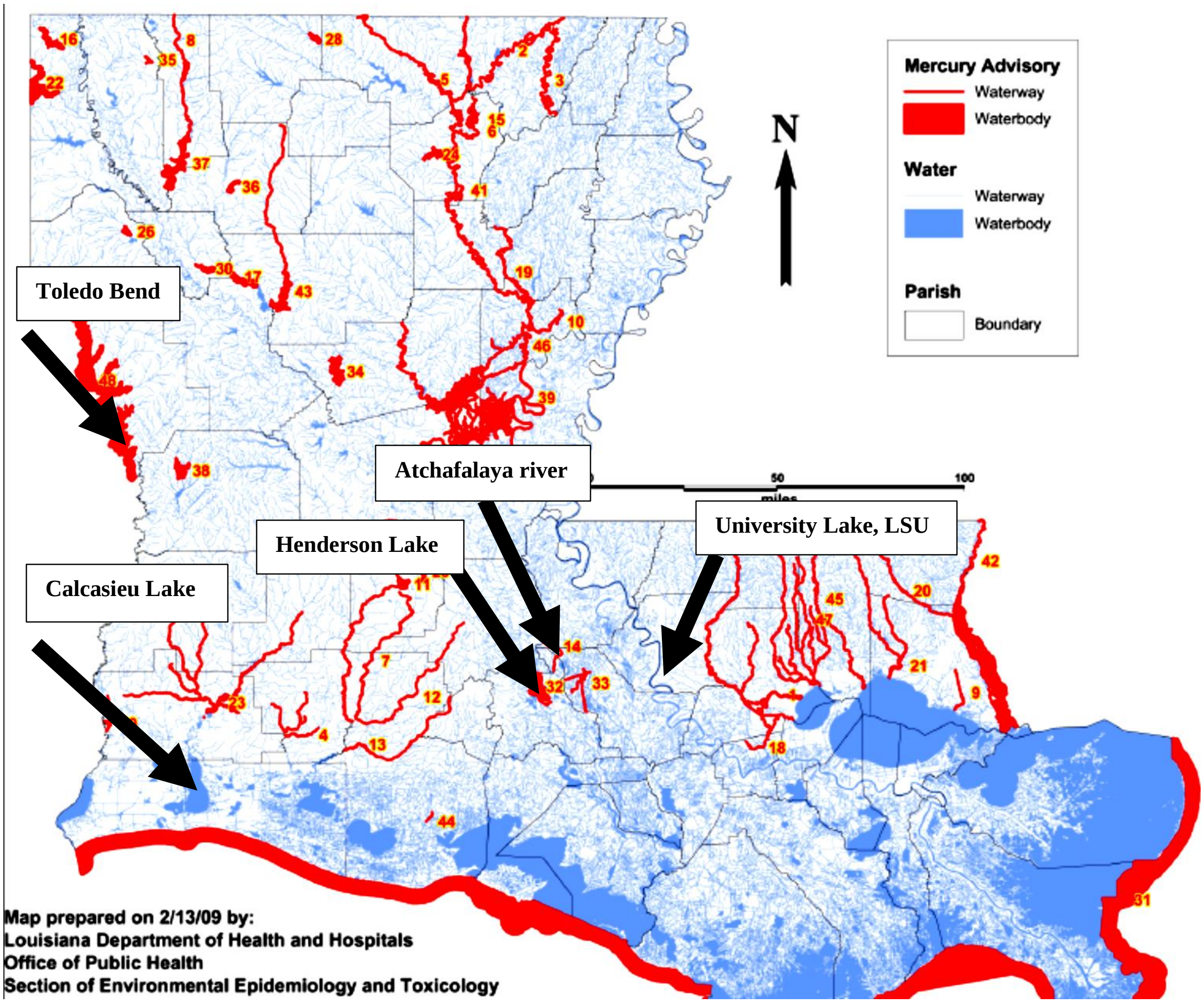

Figure 1. Sites of fish sampling in Louisiana (LDEQ 2009).

3. RESULTS AND DISCUSSION

3.1 Total Mercury Analysis.

Table 3 summarize the results of the total mercury analysis. Blanks were analyzed and reported insignificant concentrations of Hg of almost zero ppm.

Table 3. Results of total Hg in fish. dup.* = duplicate, w/w= filet wet weight basis.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Hg (nano grams)	Hg (ppm)
Black Drum	Calcasieu Lake	1	185.94	n/a	0.0533	7.7176	**0.145**
Black Drum	Calcasieu Lake	1 dup*	185.92	n/a	0.0539	7.4537	**0.138**
Black Drum	Calcasieu Lake	2	113.78	n/a	0.0565	16.8307	**0.298**
Black Drum	Calcasieu Lake	3	125.58	n/a	0.0498	10.3762	**0.208**
Black Drum	Calcasieu Lake	4	130.05	n/a	0.0525	8.953	**0.170**
Black Drum	Calcasieu Lake	5	150.34	n/a	0.0569	8.9857	**0.158**
Black Drum	Calcasieu Lake	5 dup.	150.37	n/a	0.0522	6.4017	**0.123**
Black Drum	Calcasieu Lake	6	178.42	n/a	0.0519	3.8141	**0.073**
Black Drum	Calcasieu Lake	7	105.73	n/a	0.0518	16.3882	**0.316**
Black Drum	Calcasieu Lake	8	93.99	n/a	0.0588	8.4099	**0.143**
Black Drum	Calcasieu Lake	9	110.80	n/a	0.0581	4.4364	**0.075**
Black Drum	Calcasieu Lake	10	90.97	n/a	0.0518	6.7226	**0.130**
Black Drum	Calcasieu Lake	10 dup	90.93	n/a	0.0575	6.9152	**0.120**
Cat Fish	Toledo Bend	1	84.71	n/a	0.0552	4.3262	**0.078**
Cat Fish	Toledo Bend	1 dup.	84.71	n/a	0.0554	4.7897	**0.086**
Cat Fish	Toledo Bend	2	134.24	n/a	0.0535	4.1341	**0.077**
Cat Fish	Toledo Bend	3	120.31	n/a	0.0554	8.4099	**0.152**
Cat Fish	Toledo Bend	4	108.28	n/a	0.051	2.4467	**0.048**
Cat Fish	Toledo Bend	5	91.57	n/a	0.055	3.4124	**0.062**
Cat Fish	Toledo Bend	6	113.09	n/a	0.0571	9.4371	**0.165**
Cat Fish	Toledo Bend	7	186.99	n/a	0.0527	3.5534	**0.067**
Cat Fish	Toledo Bend	8	131.88	n/a	0.053	2.2874	**0.043**
Cat Fish	Toledo Bend	9	96.71	n/a	0.0571	3.0022	**0.052**
Cat Fish	Toledo Bend	10	156.5	n/a	0.0505	2.1378	**0.042**
Cat Fish	Toledo Bend	10 dup	156.6	n/a	0.0517	2.3872	**0.046**
Large Mouth Bass	Atchafalaya	1	157.33	33.66	0.0566	9.7385	**0.172**
Large Mouth Bass	Atchafalaya	1 dup.	157.33	34.29	0.0531	9.8993	**0.186**
Large Mouth Bass	Atchafalaya	2	163.36	30.48	0.0526	18.5036	**0.352**
Large	Atchafalaya	3	141.70	34.29	0.0535	9.1545	**0.171**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Hg (nano grams)	Hg (ppm)
Mouth Bass							
Large Mouth Bass	Atchafalaya	4	216.89	35.56	0.0516	13.6741	**0.265**
Large Mouth Bass	Atchafalaya	5	171.16	27.94	0.0547	15.4973	**0.283**
Large Mouth Bass	Atchafalaya	5 dup.	171.16	30.48	0.0563	16.1281	**0.286**
Large Mouth Bass	Atchafalaya	6	117.18	38.1	0.0539	11.3138	**0.210**
Large Mouth Bass	Atchafalaya	7	135.36	31.75	0.0525	13.057	**0.249**
Large Mouth Bass	Atchafalaya	8	277.33	31.75	0.0585	21.7528	**0.372**
Large Mouth Bass	Atchafalaya	9	148.55	31.75	0.0532	9.4936	**0.179**
Large Mouth Bass	Atchafalaya	10	143.94	12.7	0.0536	10.5595	**0.197**
Large Mouth Bass	Atchafalaya	10 dup.	143.94	15.88	0.0518	10.0491	**0.194**
Blue Gill	University Lake LSU	1	41.26	20.32	0.0526	4.934	**0.094**
Blue Gill	University Lake LSU	2	69.79	19.05	0.0561	8.5789	**0.153**
Large Mouth Bass	University Lake LSU	3	118.70	12.06	0.05	9.7277	**0.195**
Large Mouth Bass	University Lake LSU	4	83.60	16.51	0.0578	7.9356	**0.137**
Blue Gill	University Lake LSU	5	31.28	25.4	0.0523	2.6525	**0.051**
Blue Gill	University Lake LSU	5 dup.	31.28	12.7	0.0554	2.8654	**0.052**
Blue Gill	University Lake LSU	6	105.16	15.24	0.056	2.8287	**0.051**
Brown Bullhead Catfish	University Lake LSU	7	344.58	13.97	0.0566	6.608	**0.117**
Blue Gill	University Lake LSU	8	38.29	13.97	0.0536	5.3456	**0.099**
Blue Gill	University Lake LSU	9	68.31	15.88	0.0535	2.9497	**0.055**
Blue Gill	University Lake LSU	10	59.56	17.78	0.0548	3.4111	**0.062**
Blue Gill	University Lake	10 dup	59.56	13.97	0.0577	3.3515	**0.058**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Hg (nano grams)	Hg (ppm)
	LSU						
Blue Gill	University Lake LSU	11	49.42	13.33	0.0571	4.6254	**0.081**
Large Mouth Bass	University Lake LSU	12	51.53	15.87	0.0549	4.8507	**0.088**
Large Mouth Bass	University Lake LSU	13	74.78	17.78	0.0513	6.5414	**0.128**
Blue Gill	University Lake LSU	14	43.82	17.78	0.0525	4.8662	**0.093**
Blue Gill	University Lake LSU	15	45.71	45.72	0.0544	4.218	**0.078**
Blue Gill	University Lake LSU	15 dup	45.71	38.1	0.0517	3.6192	**0.070**
Gizzard Shad	University Lake LSU	16	61.72	30.48	0.051	0.3236	**0.006**
Gizzard Shad	University Lake LSU	17	59.11	30.48	0.0578	0.4305	**0.007**
Large Mouth Bass	Henderson Lake, Breaux Bridge	1	101.27	33.02	0.0572	37.5088	**0.660**
Large Mouth Bass	Henderson Lake, Breaux Bridge	1 dup.	101.27	30.48	0.0554	36.3948	**0.657**
Large Mouth Bass	Henderson Lake, Breaux Bridge	2	51.05	35.56	0.0544	33.9466	**0.624**
Large Mouth Bass	Henderson Lake, Breaux Bridge	3	30.80	27.94	0.0503	22.7475	**0.452**
Large Mouth Bass	Henderson Lake, Breaux Bridge	4	18.44	27.94	0.0522	35.1703	**0.674**
Large Mouth Bass	Henderson Lake, Breaux Bridge	5	25.38	33.02	0.0566	31.8347	**0.563**
Large Mouth Bass	Henderson Lake, Breaux Bridge	5 dup.	25.38	33.02	0.0563	28.6159	**0.508**
Large Mouth Bass	Henderson Lake, Breaux Bridge	6	20.08	33.66	0.0505	27.5073	**0.544**
Large Mouth Bass	Henderson Lake, Breaux	7	25.42	34.29	0.057	36.6176	**0.642**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Hg (nano grams)	Hg (ppm)
	Bridge						
Large Mouth Bass	Henderson Lake, Breaux Bridge	8	16.12	30.48	0.0528	22.8657	**0.433**
Large Mouth Bass	Henderson Lake, Breaux Bridge	9	17.29	34.29	0.0564	27.8398	**0.494**
Large Mouth Bass	Henderson Lake, Breaux Bridge	10	21.03	35.56	0.0535	26.7316	**0.499**
Large Mouth Bass	Henderson Lake, Breaux Bridge	10 dup	21.03	27.94	0.0573	28.2832	**0.494**

3.2. Selenium Analysis

Table 4 summarize the results of the Selenium analysis. Three blanks were analyzed and reported zero ppm of the analyte.

Table 4. Results of Se analysis in fish. dup.* = duplicate, w/w= filet wet weight basis.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Se 78 (ppm)
Black Drum	Calcasieu Lake	1	185.94	n/a	1.05	**0.886**
Black Drum	Calcasieu Lake	2	113.78	n/a	1.07	**0.636**
Black Drum	Calcasieu Lake	3	125.58	n/a	1.02	**0.241**
Black Drum	Calcasieu Lake	4	130.05	n/a	1.04	**0.584**
Black Drum	Calcasieu Lake	5	150.34	n/a	1.01	**0.545**
Black Drum	Calcasieu Lake	6	178.42	n/a	1.04	**0.808**
Black Drum	Calcasieu Lake	7	105.73	n/a	1.01	**0.78**
Black Drum	Calcasieu Lake	8	93.99	n/a	1.01	**0.475**
Black Drum	Calcasieu Lake	9	110.80	n/a	1.01	**0.409**
Black Drum	Calcasieu Lake	10	90.97	n/a	1.05	**0.561**
Black Drum	Calcasieu Lake	10 dup	90.93	n/a	1.04	**0.353**
Cat Fish Sp	Toledo Bend	1	84.71	n/a	1.07	**0.093**
Cat Fish	Toledo Bend	2	134.24	n/a	1.02	**0.068**
Cat Fish	Toledo Bend	3	120.31	n/a	1.07	**0.096**
Cat Fish	Toledo Bend	4	108.28	n/a	1.01	**0.093**
Cat Fish	Toledo Bend	5	91.57	n/a	1.05	**0.104**

table cont'd.

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Se 78 (ppm)
Cat Fish	Toledo Bend	6	113.09	n/a	1.06	**0.087**
Cat Fish	Toledo Bend	7	186.99	n/a	1.06	**0.152**
Cat Fish	Toledo Bend	8	131.88	n/a	1.03	**0.121**
Cat Fish	Toledo Bend	9	96.71	n/a	1.07	**0.074**
Cat Fish	Toledo Bend	10	156.5	n/a	1.06	**0.071**
Cat Fish	Toledo Bend	10 dup	156.6	n/a	1.02	**0.094**
Large Mouth Bass	Atchafalaya	1	157.33	33.66	1.01	**0.16**
Large Mouth Bass	Atchafalaya	2	163.36	34.29	1.06	**0.199**
Large Mouth Bass	Atchafalaya	3	141.70	30.48	1	**0.076**
Large Mouth Bass	Atchafalaya	4	216.89	34.29	1.05	**0.152**
Large Mouth Bass	Atchafalaya	5	171.16	35.56	1.05	**0.227**
Large Mouth Bass	Atchafalaya	6	117.18	27.94	1.04	**0.165**
Large Mouth Bass	Atchafalaya	7	135.36	30.48	1.07	**0.144**
Large Mouth Bass	Atchafalaya	8	277.33	38.1	1.06	**0.096**
Large Mouth Bass	Atchafalaya	9	148.55	31.75	1.02	**0.127**
Large Mouth Bass	Atchafalaya	10	143.94	31.75	1.09	**0.186**
Large Mouth Bass	Atchafalaya	10 dup.	143.94	31.75	1.08	**0.085**
Blue Gill	University Lake LSU	1	41.26	12.7	1.02	**0.032**
Blue Gill	University Lake LSU	2	69.79	15.88	1.07	**0.042**
Large Mouth Bass	University Lake LSU	3	118.70	20.32	1.06	**0.063**
Large Mouth Bass	University Lake LSU	4	83.60	19.05	1.04	**0.048**
Blue Gill	University Lake LSU	5	31.28	12.06	1.05	**0.069**
Blue Gill	University Lake LSU	6	105.16	16.51	1.08	**0.096**
Brown Bullhead	University Lake LSU	7	344.58	25.4	1.05	**0.062**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Se 78 (ppm)
Catfish						
Blue Gill	University Lake LSU	8	38.29	12.7	1.06	**0.058**
Blue Gill	University Lake LSU	9	68.31	15.24	1.09	**0.053**
Blue Gill	University Lake LSU	10	59.56	13.97	1.06	**0.087**
Blue Gill	University Lake LSU	11	49.42	13.97	1.06	**0.067**
Large Mouth Bass	University Lake LSU	12	51.53	15.88	1.05	**0.03**
Large Mouth Bass	University Lake LSU	13	74.78	17.78	1.09	**0.034**
Blue Gill	University Lake LSU	14	43.82	13.97	1.06	**0.112**
Blue Gill	University Lake LSU	15	45.70	13.33	1.06	**0.037**
Large Mouth Bass	University Lake LSU	12 dup	51.53	15.87	1.06	**0.085**
Gizzard Shad	University Lake LSU	16	61.72	17.78	1.09	**0.089**
Gizzard Shad	University Lake LSU	17	59.11	17.78	1.09	**0.129**
Large Mouth Bass	Henderson Lake, Breaux Bridge	1	101.27	45.72	1.01	**0.099**
Large Mouth Bass	Henderson Lake, Breaux Bridge	2	51.04	38.1	1.05	**0.125**
Large Mouth Bass	Henderson Lake, Breaux Bridge	3	30.80	30.48	1.02	**0.101**
Large Mouth Bass	Henderson Lake, Breaux Bridge	4	18.44	30.48	1.04	**0.122**
Large Mouth Bass	Henderson Lake, Breaux Bridge	5	25.38	33.02	1.05	**0.114**
Large Mouth Bass	Henderson Lake, Breaux Bridge	6	20.08	30.48	1.04	**0.094**
Large Mouth Bass	Henderson Lake, Breaux Bridge	7	25.42	35.56	1.03	**0.075**
Large Mouth Bass	Henderson Lake, Breaux Bridge	8	16.12	27.94	1.07	**0.037**
Large Mouth Bass	Henderson Lake, Breaux Bridge	9	17.29	27.94	1.04	**0.114**
Large Mouth Bass	Henderson Lake, Breaux Bridge	10	21.03	33.02	1.09	**0.039**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	Se 78 (ppm)
Large Mouth Bass	Henderson Lake, Breaux Bridge	10 dup	21.03	33.02	1.03	**0.024**

3.3. Se to Hg Molar Ratios

Table 5 summarize the calculated molar ratios of Se to Hg. For this calculation, total mercury measured in ppm (µg/g of wet weight fish tissue) was converted to micromoles via atomic weight (Hg = 200.59 µg/µmol). Likewise, Se, molecular weight (Se = 78.97µmol/µg) was used to calculate the ratios.

Table 5. Se-to-Hg molar ratios. dup.* = duplicate.

Species	Location	Sample #	Se 78 µmol	Hg µmol	Se:Hg ratio
Black Drum	Calcasieu Lake	1	0.01121945	0.00072187	**15.542**
Black Drum	Calcasieu Lake	2	0.008053691	0.001485119	**5.423**
Black Drum	Calcasieu Lake	3	0.003051792	0.001038935	**2.937**
Black Drum	Calcasieu Lake	4	0.007395213	0.000849993	**8.700**
Black Drum	Calcasieu Lake	5	0.006901355	0.000787178	**8.767**
Black Drum	Calcasieu Lake	6	0.01023173	0.000365422	**27.999**
Black Drum	Calcasieu Lake	7	0.00987717	0.001577347	**6.262**
Black Drum	Calcasieu Lake	8	0.00601494	0.000712897	**8.437**
Black Drum	Calcasieu Lake	9	0.00517918	0.000375393	**13.797**
Black Drum	Calcasieu Lake	10	0.00710396	0.000647091	**10.978**
Black Drum	Calcasieu Lake	10 dup.*	0.00447005	0.000599731	**7.453**
Cat Fish	Toledo Bend	1	0.001177662	0.000390847	**3.013**
Cat Fish	Toledo Bend	2	0.00086109	0.000385363	**2.234**
Cat Fish	Toledo Bend	3	0.00121565	0.000756768	**1.606**

table cont'd.

Species	Location	Sample #	Se 78 µmol	Hg µmol	Se:Hg ratio
Cat Fish	Toledo Bend	4	0.00117766	0.000239294	**4.9214**
Cat Fish	Toledo Bend	5	0.00131696	0.000309088	**4.261**
Cat Fish	Toledo Bend	6	0.00110168	0.000824069	**1.337**
Cat Fish	Toledo Bend	7	0.00192478	0.000336009	**5.728**
Cat Fish	Toledo Bend	8	0.00153223	0.000215365	**7.114**
Cat Fish	Toledo Bend	9	0.00093706	0.000262226	**3.573**
Cat Fish	Toledo Bend	10	0.00089908	0.000210878	**4.263**
Cat Fish	Toledo Bend	10 dup	0.00119033	0.000230321	**5.168**
Large Mouth Bass	Atchafalaya	1	0.002026086	0.000857969	**2.361**
Large Mouth Bass	Atchafalaya	2	0.00251994	0.001753826	**1.437**
Large Mouth Bass	Atchafalaya	3	0.00096239	0.000852984	**1.128**
Large Mouth Bass	Atchafalaya	4	0.00192478	0.001321103	**1.457**
Large Mouth Bass	Atchafalaya	5	0.00287451	0.001412334	**2.035**
Large Mouth Bass	Atchafalaya	6	0.0020894	0.001046413	**1.997**
Large Mouth Bass	Atchafalaya	7	0.00182348	0.001239842	**1.471**
Large Mouth Bass	Atchafalaya	8	0.00121565	0.001853532	**0.656**
Large Mouth Bass	Atchafalaya	9	0.00160821	0.000889875	**1.807**
Large Mouth Bass	Atchafalaya	10	0.00235532	0.000982103	**2.398**
Large Mouth Bass	Atchafalaya	10 dup.	0.00107636	0.000967147	**1.113**
Blue Gill	University Lake LSU	1	0.000405217	0.000467621	**0.867**
Blue Gill	University Lake LSU	2	0.00053185	0.000762251	**0.698**
Large Mouth Bass	University Lake LSU	3	0.00079777	0.000970138	**0.822**
Large Mouth Bass	University Lake LSU	4	0.00060783	0.000684481	**0.888**
Blue Gill	University Lake LSU	5	0.00087375	0.000252754	**3.457**
Blue Gill	University Lake LSU	6	0.00121565	0.000251757	**4.829**
Brown Bullhead	University	7	0.00078511	0.000581784	**1.349**

table cont'd.

Species	Location	Sample #	Se 78 µmol	Hg µmol	Se:Hg ratio
Catfish	Lake LSU				
Blue Gill	University Lake LSU	8	0.00073446	0.000497034	**1.477**
Blue Gill	University Lake LSU	9	0.00067114	0.00027469	**2.443**
Blue Gill	University Lake LSU	10	0.00110168	0.000310085	**3.553**
Blue Gill	University Lake LSU	11	0.00084842	0.000403809	**2.101**
Large Mouth Bass	University Lake LSU	12	0.00037989	0.0004407	**0.862**
Large Mouth Bass	University Lake LSU	13	0.00043054	0.000635625	**0.677**
Blue Gill	University Lake LSU	14	0.00141826	0.000462137	**3.069**
Blue Gill	University Lake LSU	15	0.00046853	0.00038636	**1.213**
Large Mouth Bass	University Lake LSU	12 dup	0.001076358	0.0004407	**2.442**
Gizzard Shad	University Lake LSU	16	0.00112701	3.14073E-05	**35.884**
Gizzard Shad	University Lake LSU	17	0.00163353	3.68912E-05	**44.280**
Large Mouth Bass	Henderson Lake, Breaux Bridge	1	0.001253641	0.003268857	**0.384**
Large Mouth Bass	Henderson Lake, Breaux Bridge	2	0.00158288	0.003110823	**0.509**
Large Mouth Bass	Henderson Lake, Breaux Bridge	3	0.00127897	0.00225435	**0.567**
Large Mouth Bass	Henderson Lake, Breaux Bridge	4	0.00154489	0.003359091	**0.460**
Large Mouth Bass	Henderson Lake, Breaux Bridge	5	0.00144359	0.002804228	**0.515**
Large Mouth Bass	Henderson Lake, Breaux Bridge	6	0.00119033	0.002715489	**0.438**
Large Mouth Bass	Henderson Lake, Breaux	7	0.00094973	0.003202552	**0.297**

table cont'd.

Species	Location	Sample #	Se 78 µmol	Hg µmol	Se:Hg ratio
	Bridge				
Large Mouth Bass	Henderson Lake, Breaux Bridge	8	0.00046853	0.002159131	**0.217**
Large Mouth Bass	Henderson Lake, Breaux Bridge	9	0.00144359	0.002460741	**0.586**
Large Mouth Bass	Henderson Lake, Breaux Bridge	10	0.00049386	0.002491151	**0.198**
Large Mouth Bass	Henderson Lake, Breaux Bridge	10 dup	0.00030391	0.002460741	**0.123**

3.4. Statistical Analysis of Results

3.4.1. Black drum from Calcasieu Lake

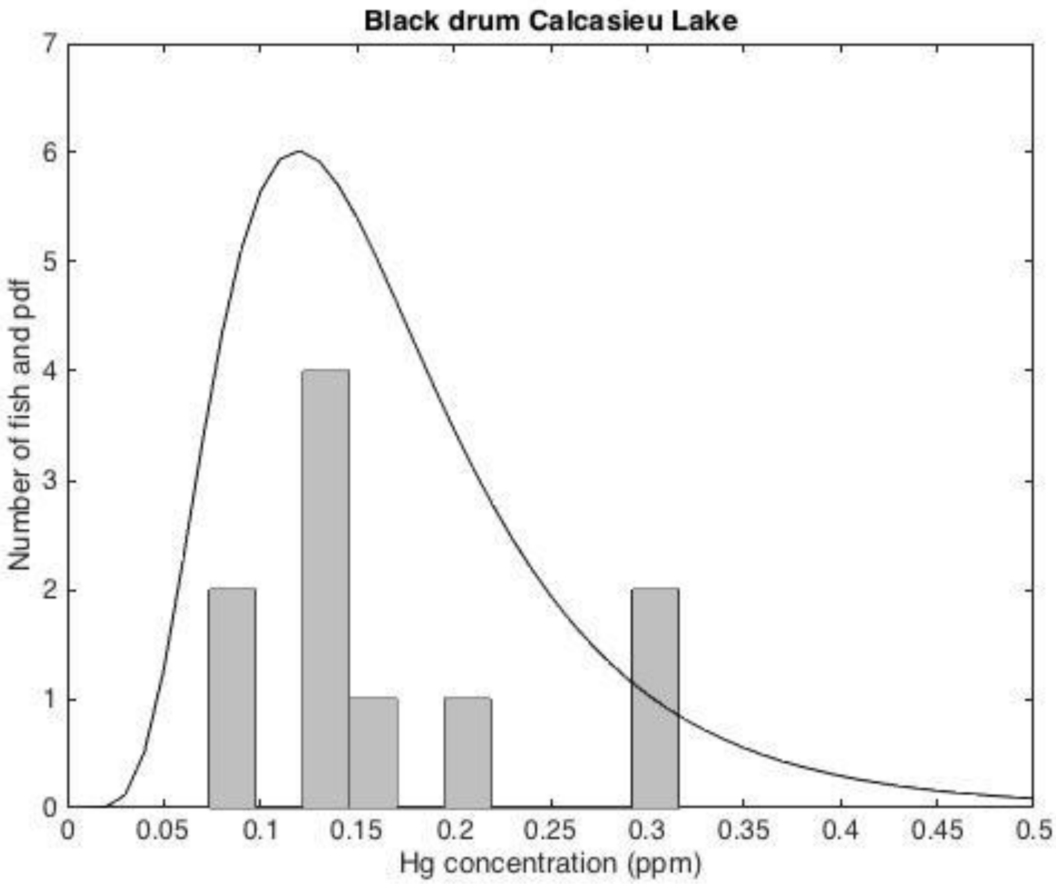

Figure 2. Hg in Black Drum.

I assumed that the Black Drum total Hg concentrations were log-normally distributed. I tested the logarithms for normality with a Kolmogorov–Smirnov (KS) test. Figure 2 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.4859, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 0.9996.

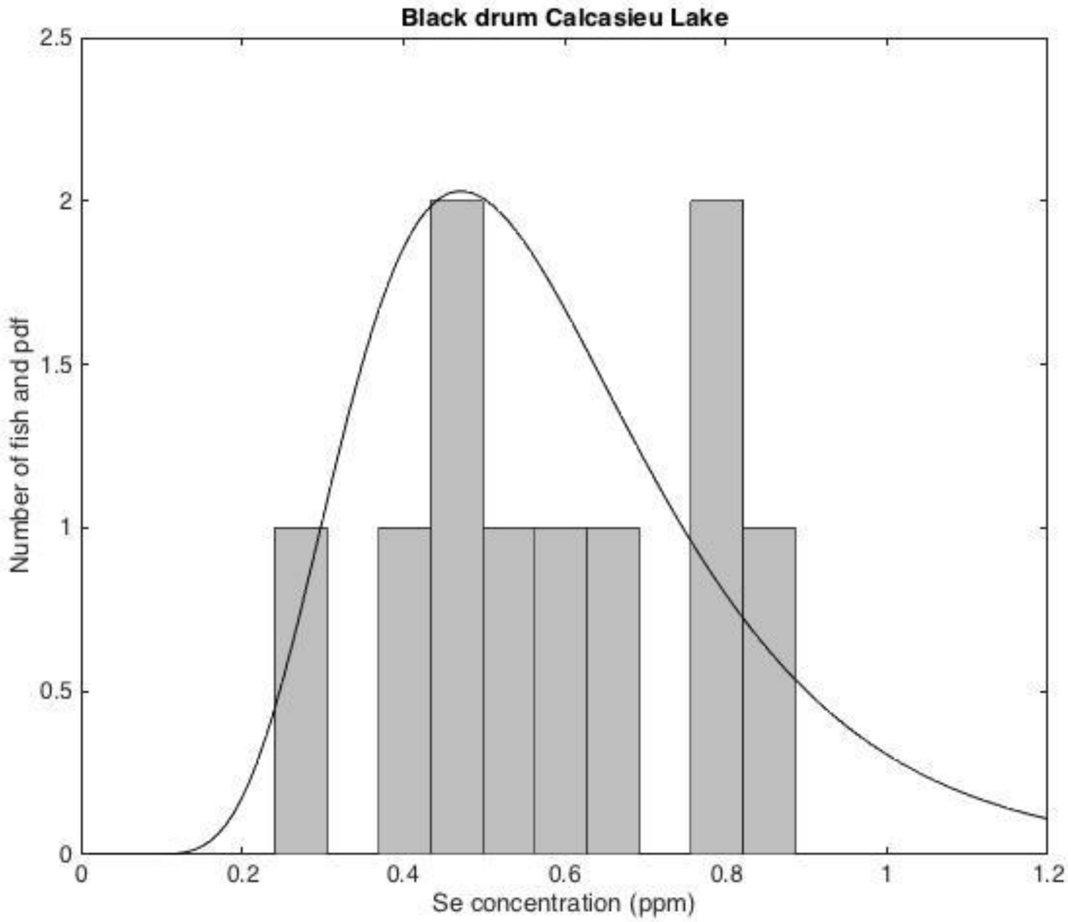

Figure 3. Se in Black Drum

I assumed that the Black Drum Se concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 3 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.9855, and I therefore accepted the null hypothesis that the data were log-normally distributed.

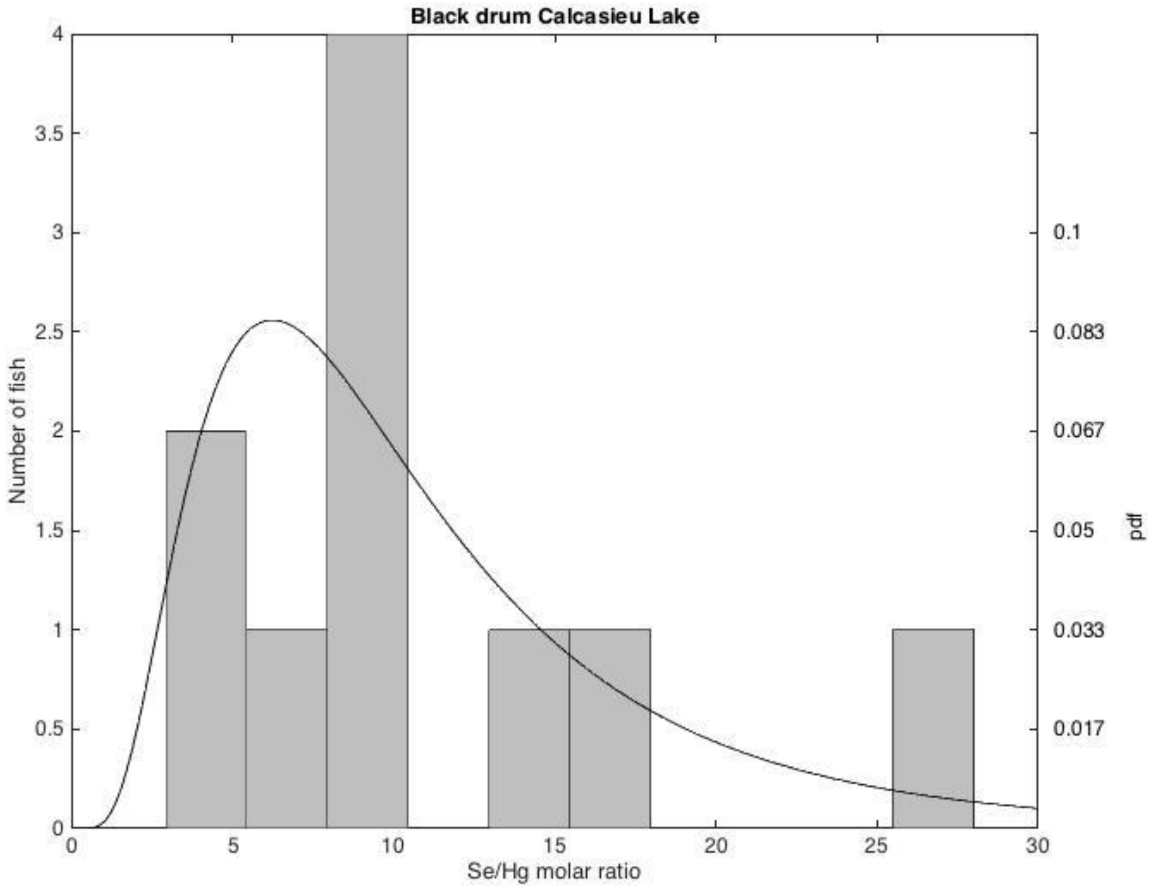

Figure 4. Se:Hg molar ratio in Black Drum

I assumed that the Black Drum Se:Hg ratios were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 4 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.4734, and I therefore accepted the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.002.

3.4.2. Catfish from Toledo Bend

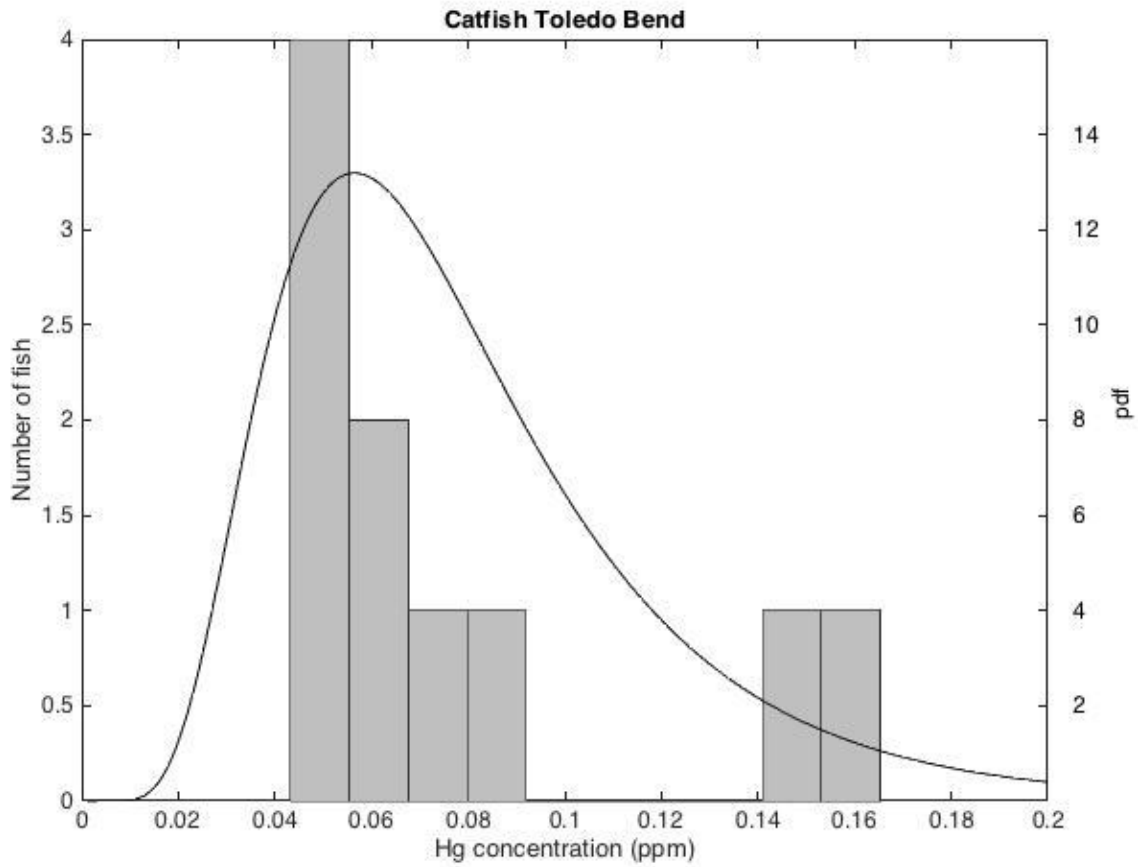

Figure 5. Hg in Catfish

I assumed that the catfish total Hg concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 5 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.4299, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 0.9999.

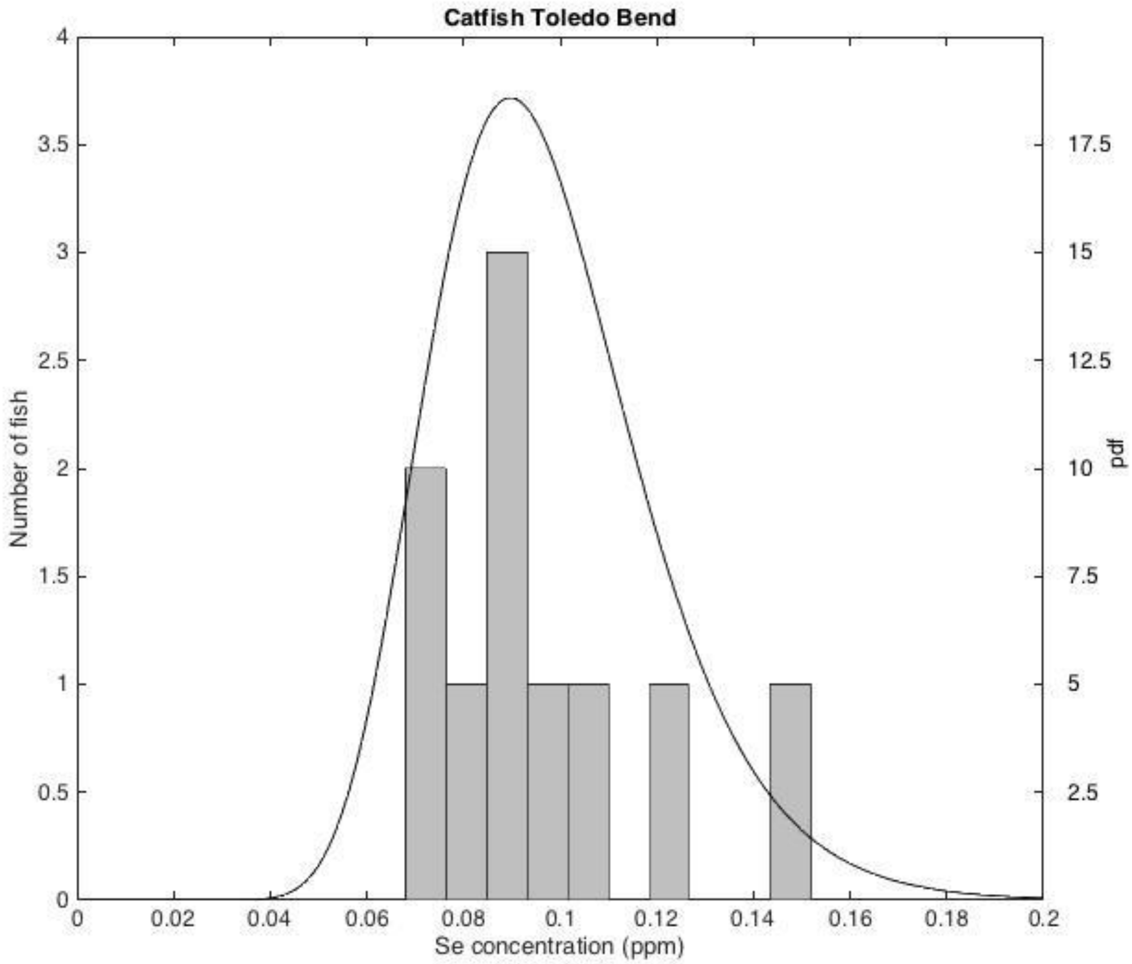

Figure 6. Se in Catfish

I assumed that the catfish Se concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 6 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.5720, and I therefore accepted the null hypothesis that the data were log-normally distributed.

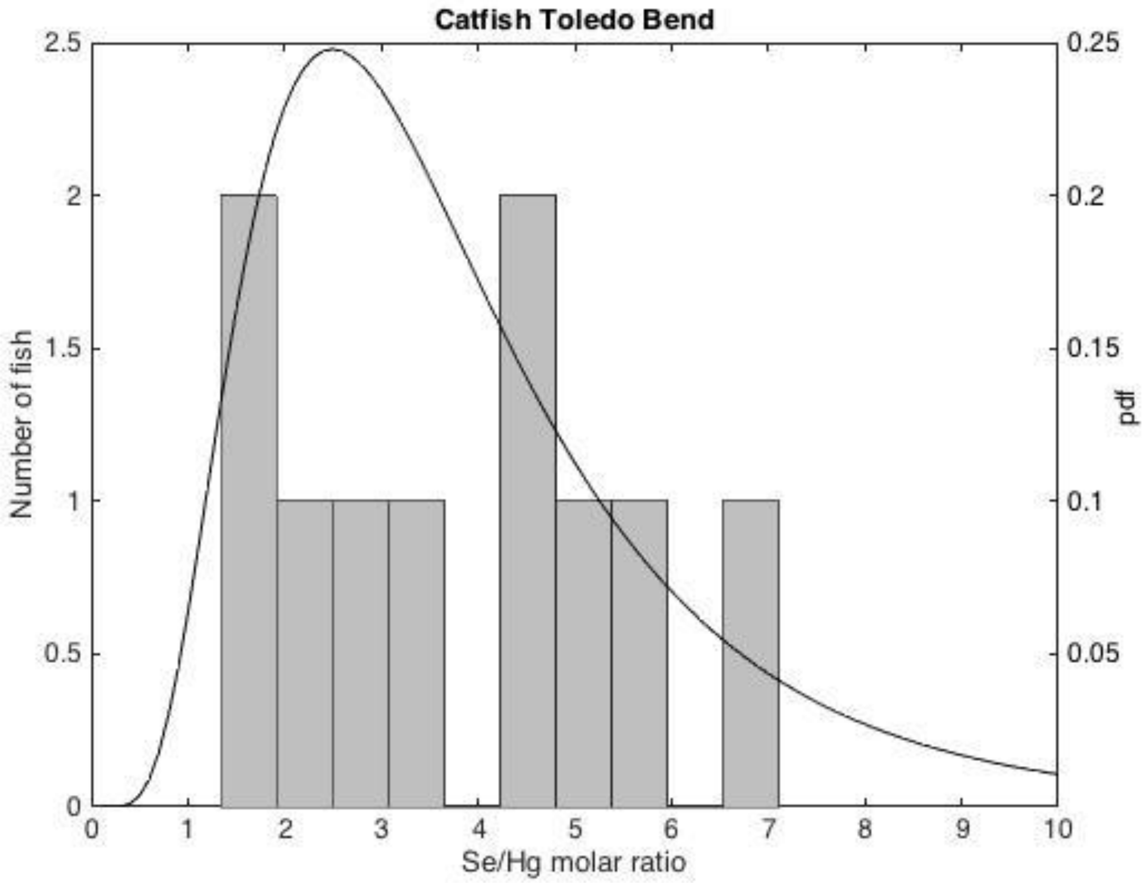

Figure 7. Se:Hg molar ratio in Catfish

I assumed that the catfish Se:Hg ratios were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 7 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.9924, and I therefore accepted the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.0204.

3.4.3. Largemouth bass from Atchafalaya River

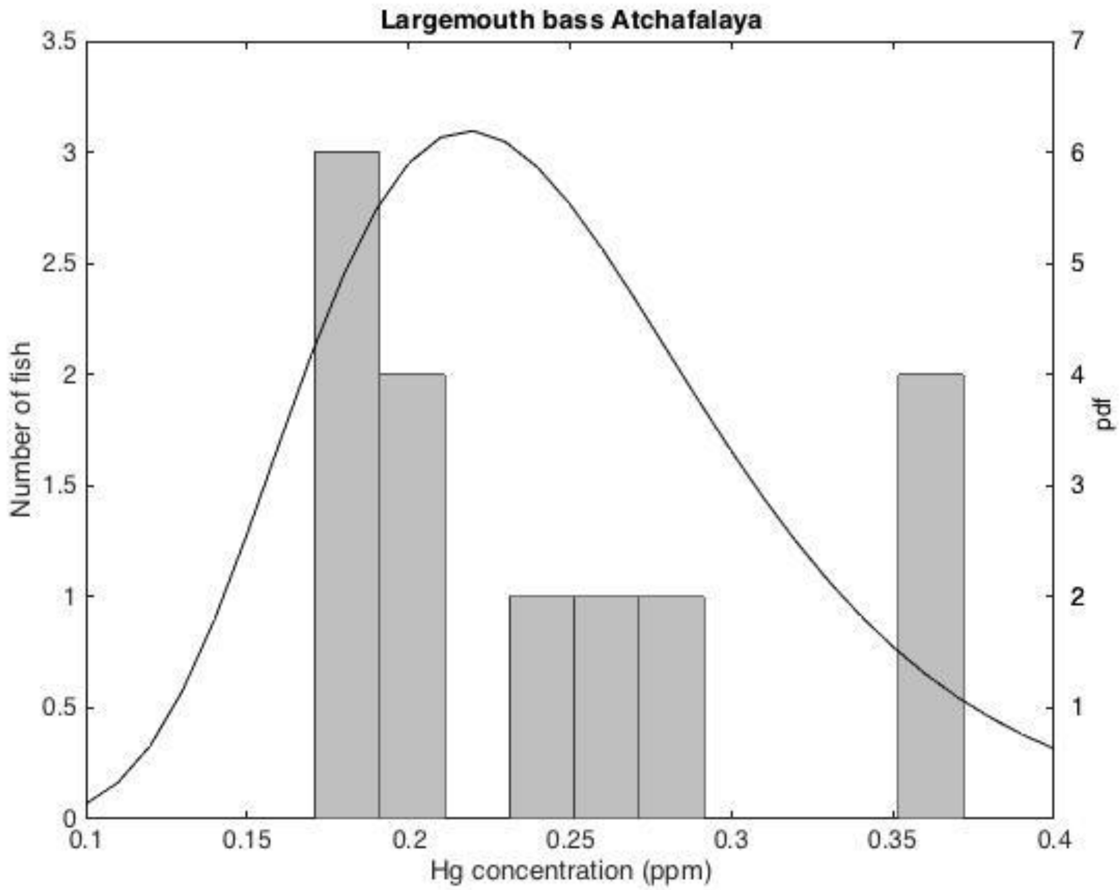

Figure 8. Hg in Largemouth Bass Atchafalaya

I assumed that the Atchafalaya largemouth bass total Hg concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 8 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.5607, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 0.999.

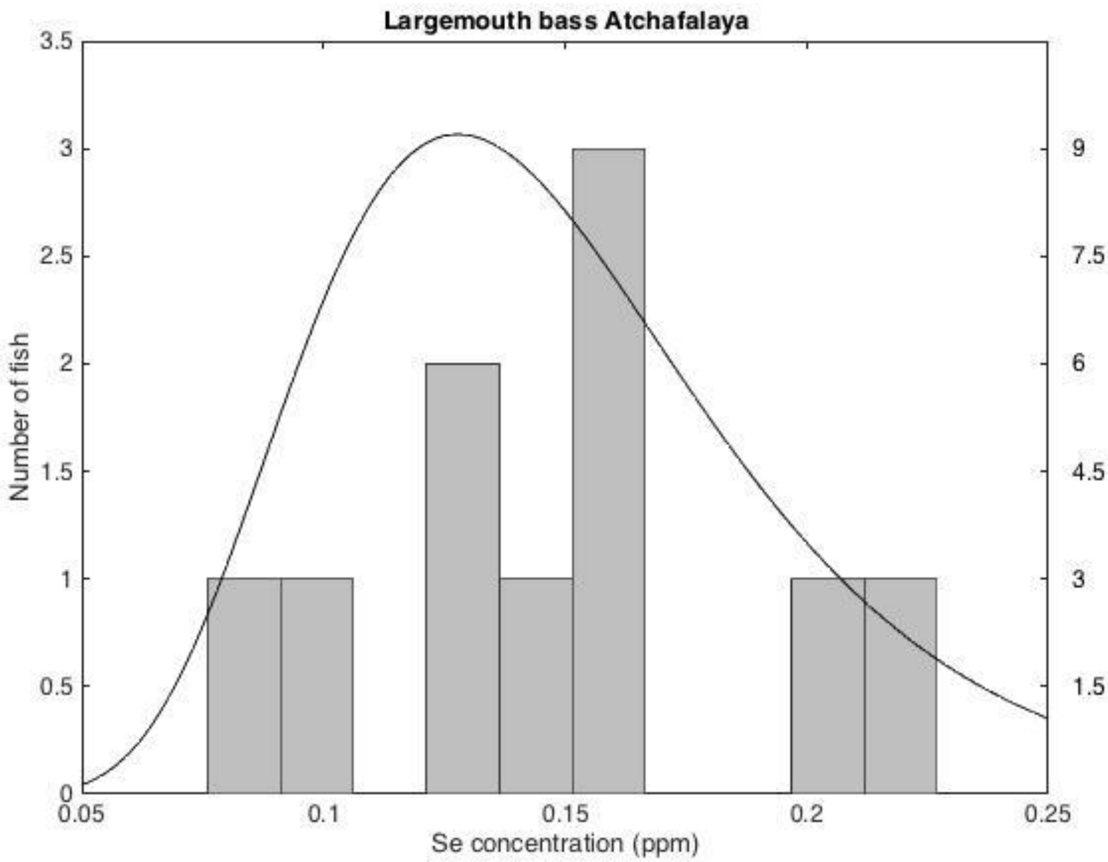

Figure 9. Se in Largemouth Bass Atchafalaya

I assumed that the Atchafalaya largemouth bass Se concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 9 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.9823, and I therefore accepted the null hypothesis that the data were log-normally distributed.

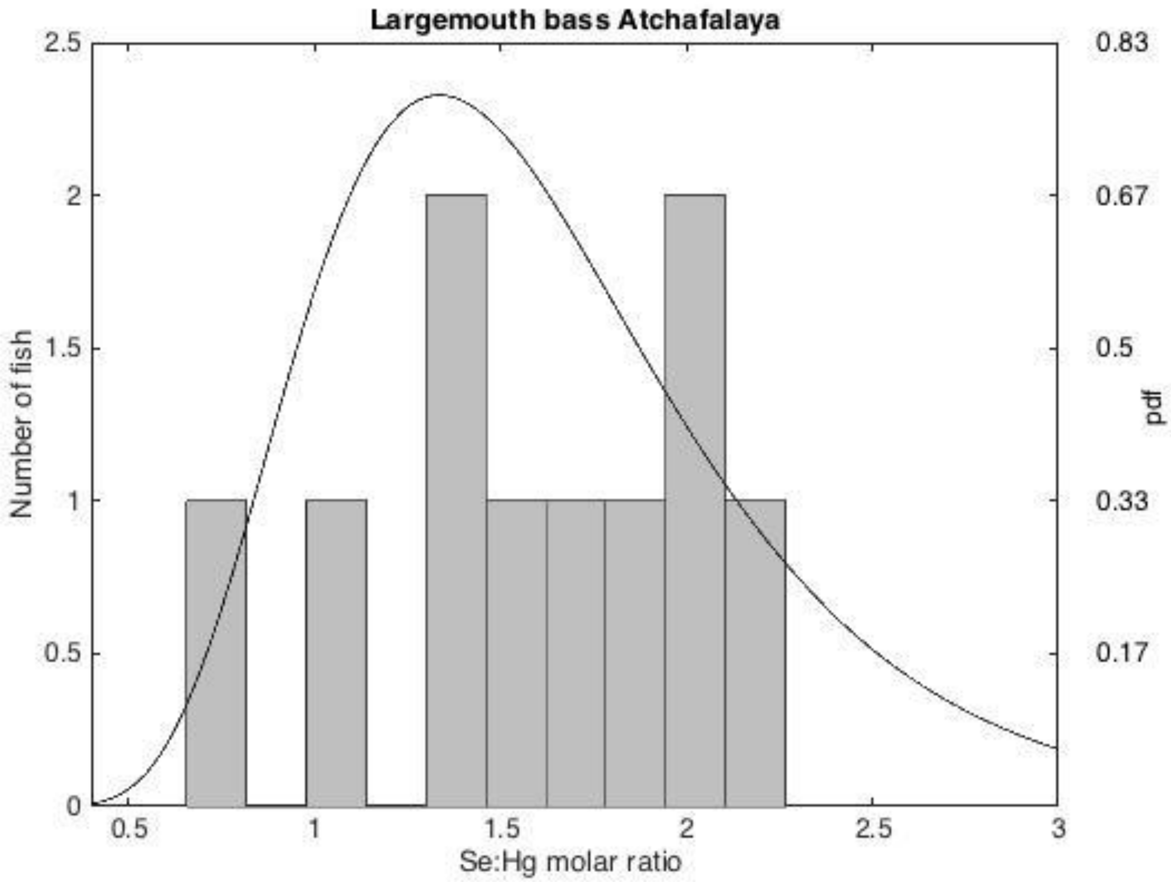

Figure 10. Se:Hg molar ratio in Largemouth Bass Atchafalaya

I assumed that the Atchafalaya largemouth bass Se:Hg ratios were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 10 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.9180, and I therefore accepted the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.1492.

3.4.4. Largemouth bass from Henderson Lake

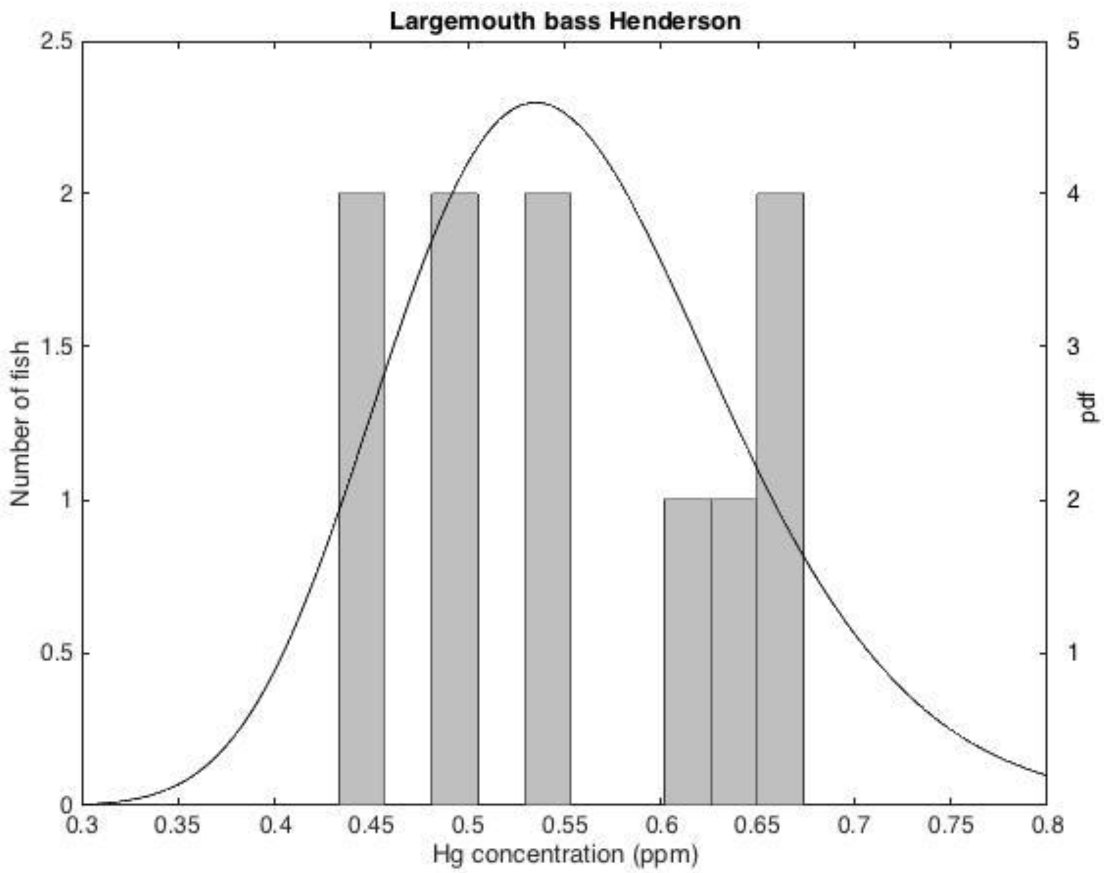

Figure 11. Hg in Largemouth Bass Henderson

I assumed that the Henderson largemouth bass total Hg concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 11 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.7327, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 0.999.

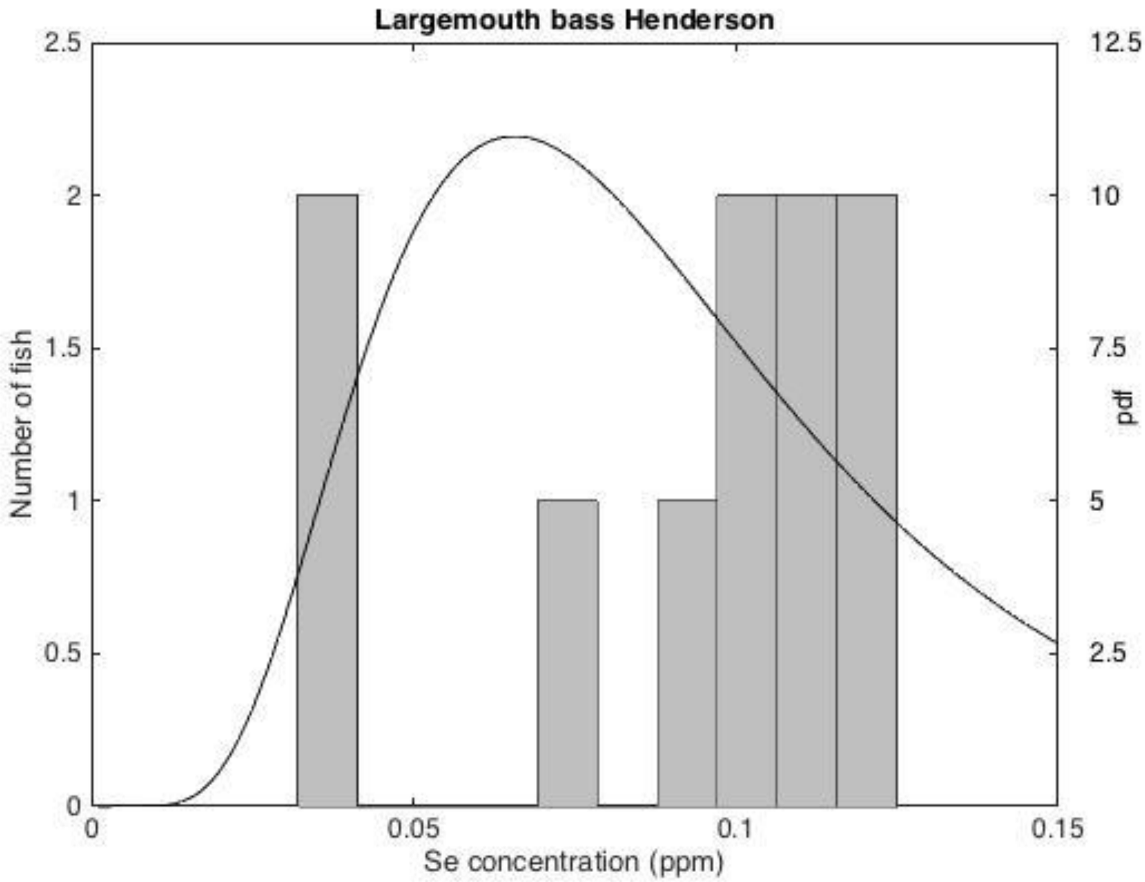

Figure 12. Se in Largemouth Bass Henderson

I assumed that the Henderson largemouth bass Se concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 12 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.5610, and I therefore accepted the null hypothesis that the data were log-normally distributed.

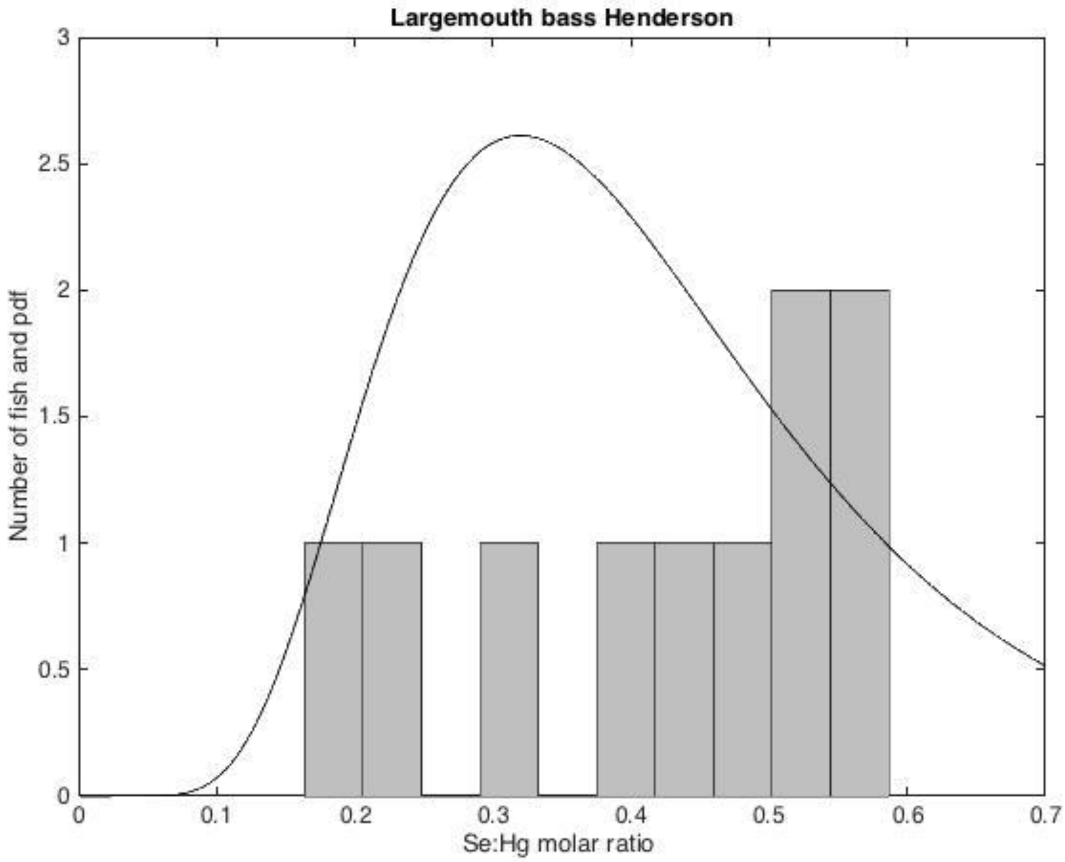

Figure 13. Se: molar ratio Hg in Largemouth Bass Henderson

I assumed that the Atchafalaya largemouth bass Se:Hg ratios were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 13 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.8845, and I therefore accepted the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.9661.

3.4.5. Bluegill fish from University Lake, LSU

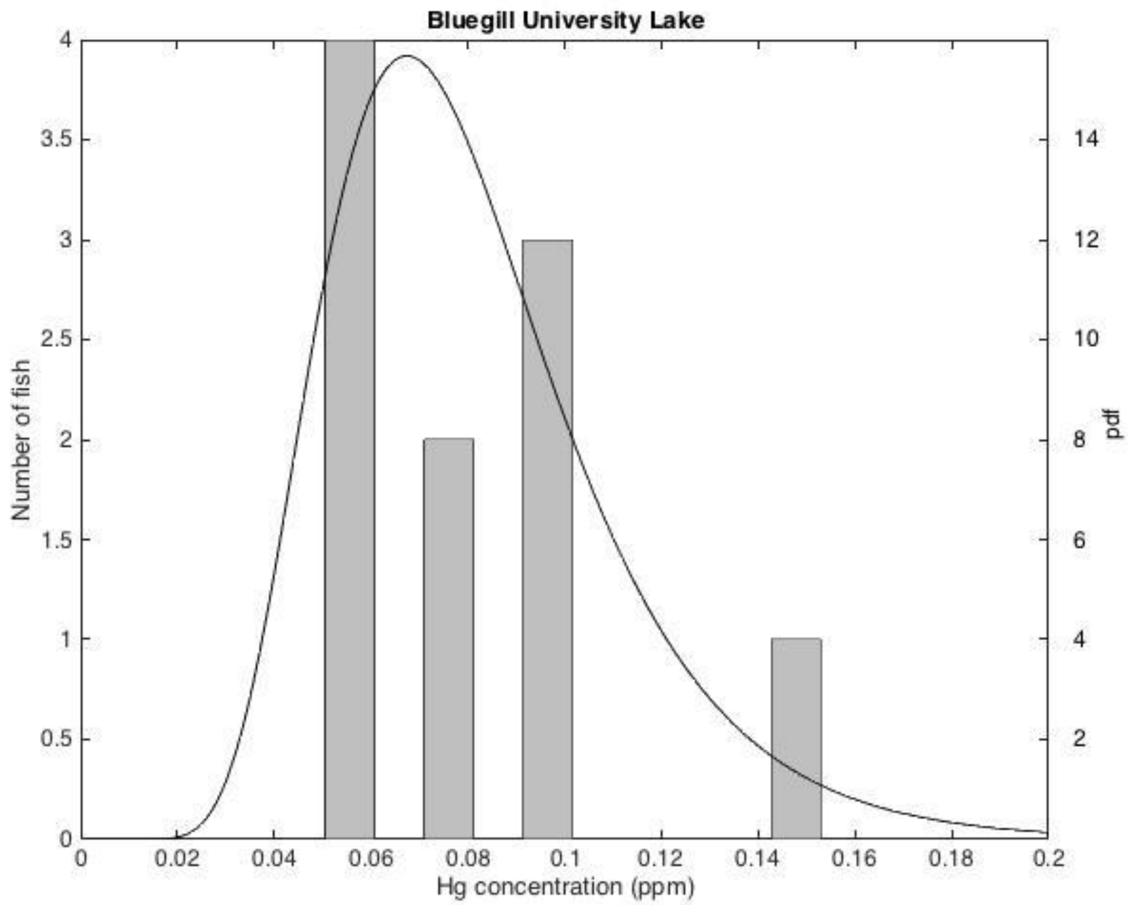

Figure 14. Hg in Bluegill from University lake

I assumed that the bluegill fish from University lake total Hg concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 14 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.7156, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 1.0.

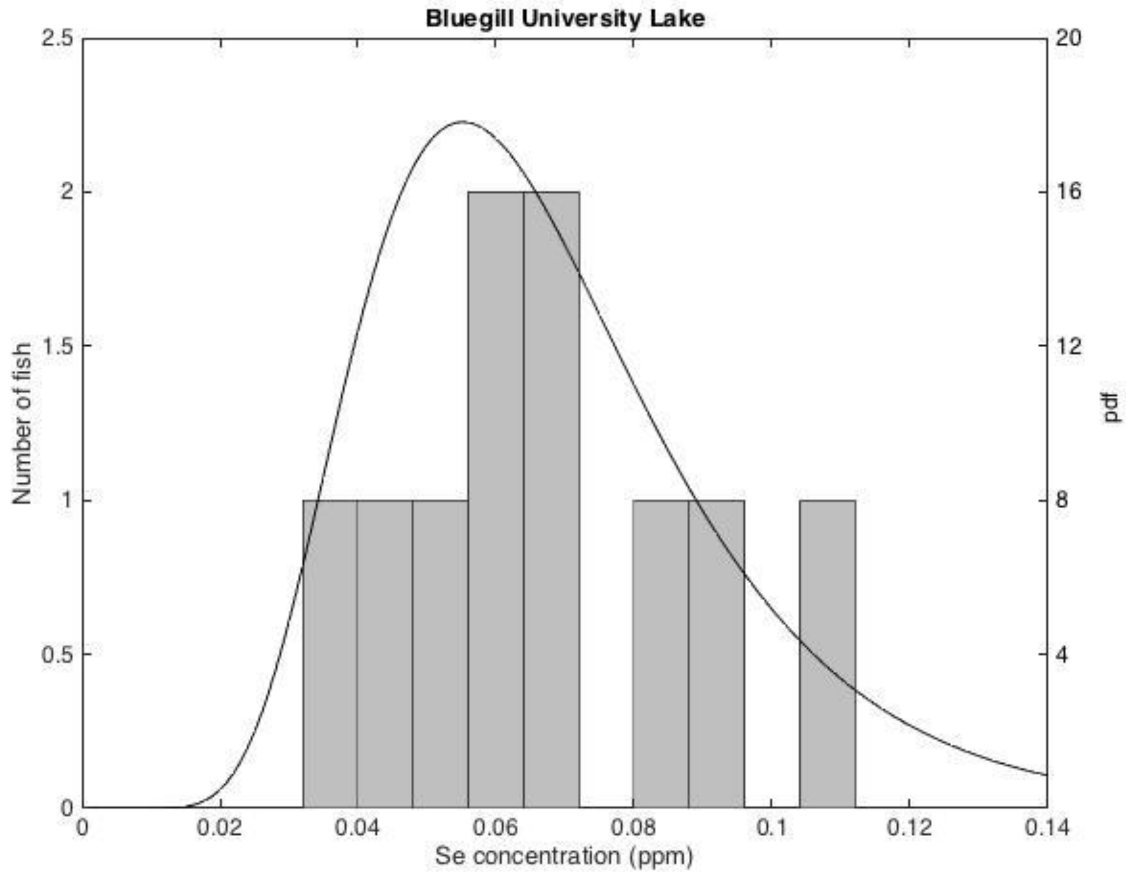

Figure 15. Se in Bluegill from University lake

I assumed that the bluegill fish from University lake Se concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 15 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.9666, and I therefore accepted the null hypothesis that the data were log-normally distributed.

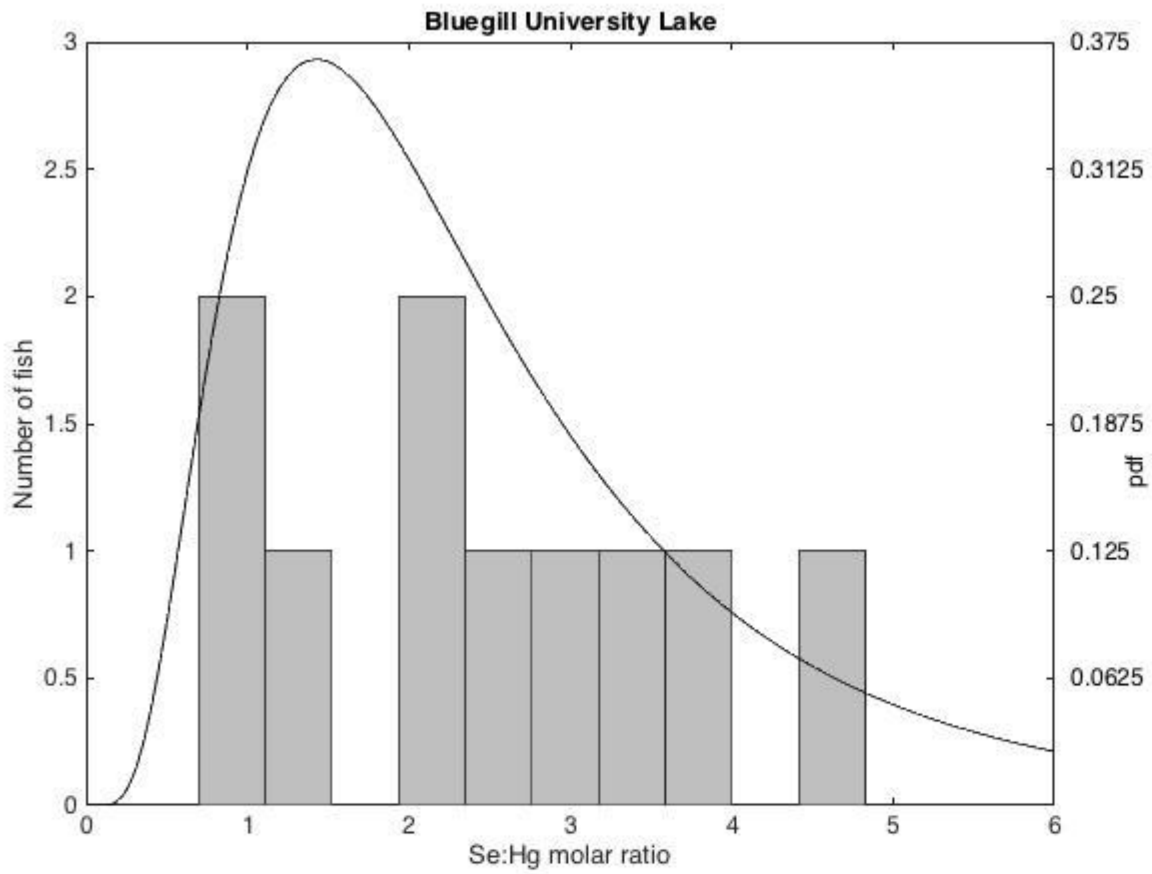

Figure 16. Se:Hg molar ratio in Bluegill from University lake

I assumed that the bluegill fish from University lake Se:Hg ratios were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 16 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.9631, and I therefore accepted the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.1568.

3.4.6. Largemouth bass from University Lake, LSU

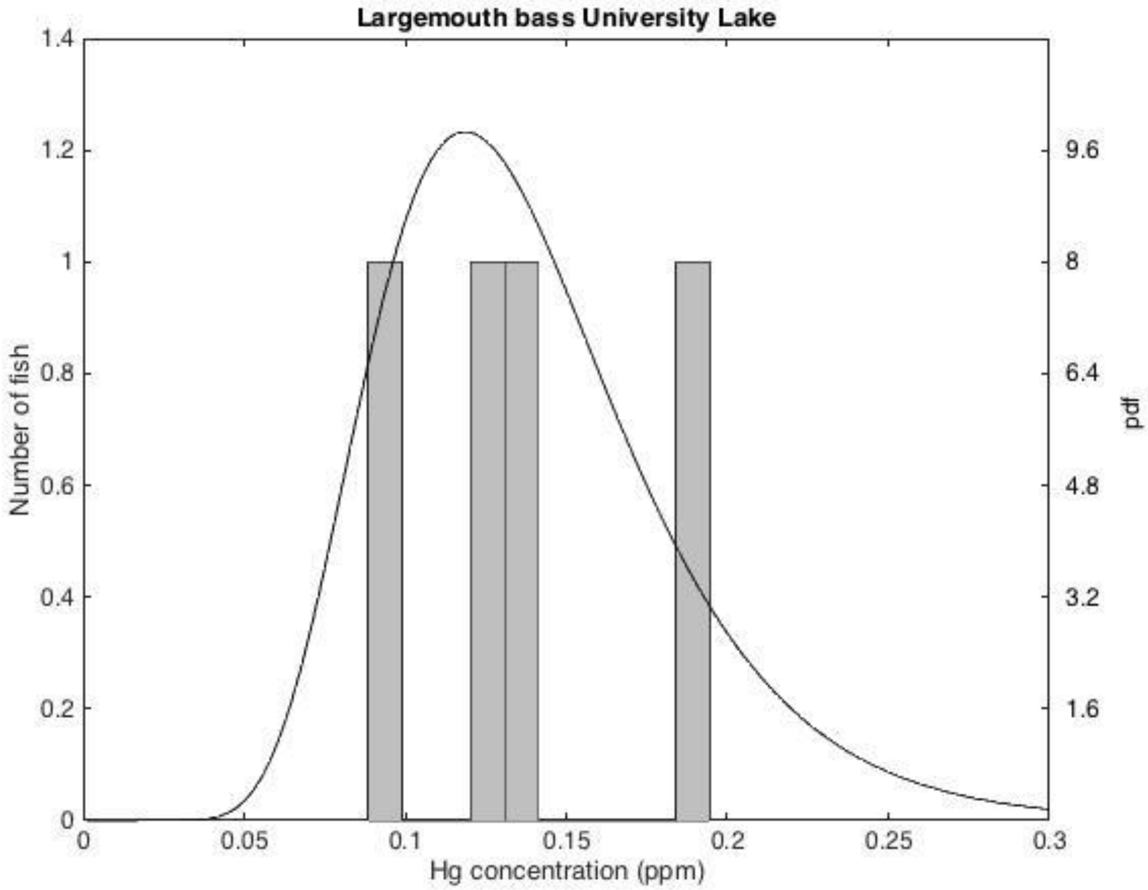

Figure 17. Hg in Largemouth Bass from University Lake

I assumed that the largemouth bass from University lake total Hg concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 17 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.9395, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 0.9984.

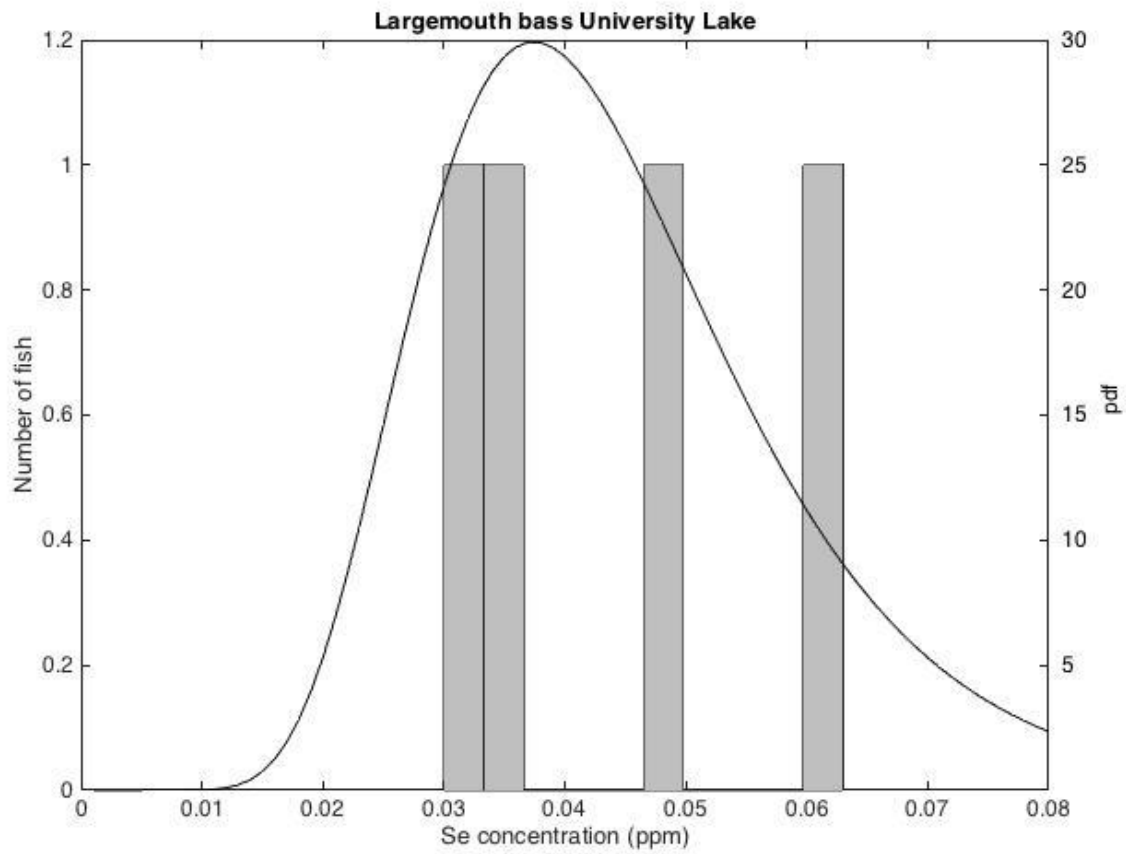

Figure 18. Se in Largemouth Bass from University Lake

I assumed that the largemouth bass from University lake Se concentrations were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 18 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.9663, and I therefore accepted the null hypothesis that the data were log-normally distributed.

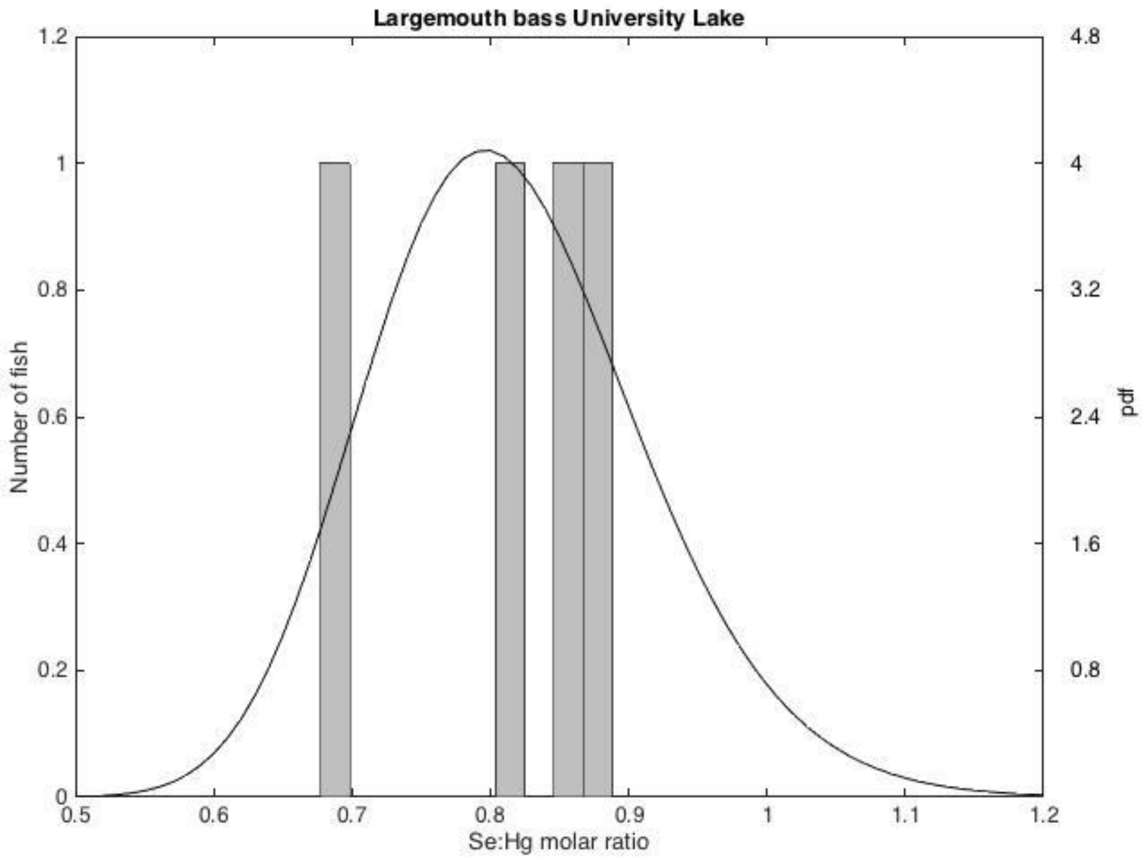

Figure 19. Se:Hg molar ratio in Largemouth Bass from University Lake

I assumed that the largemouth bass from University lake Se:Hg ratios were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 19 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.2280, and I therefore accepted the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.4940.

3.4.7. Gizzard Shad & Brown Bullhead Catfish, University Lake, LSU

Two samples of Gizzard shad and one sample of Brown Bullhead Catfish complete the set of Samples for University lake in LSU.

Table 6. Hg, Se, and Se:Hg molar ratios in 2 Species from University Lake

Species	Total Hg (ppm)	Se (ppm)	Se to Hg molar ratios
Brown Bullhead Catfish	0.1167	0.062	1.3494
Gizzard Shad	0.0063	0.089	35.883
Gizzard Shad	0.0074	0.129	44.279

3.4.8. All fish from University Lake, LSU

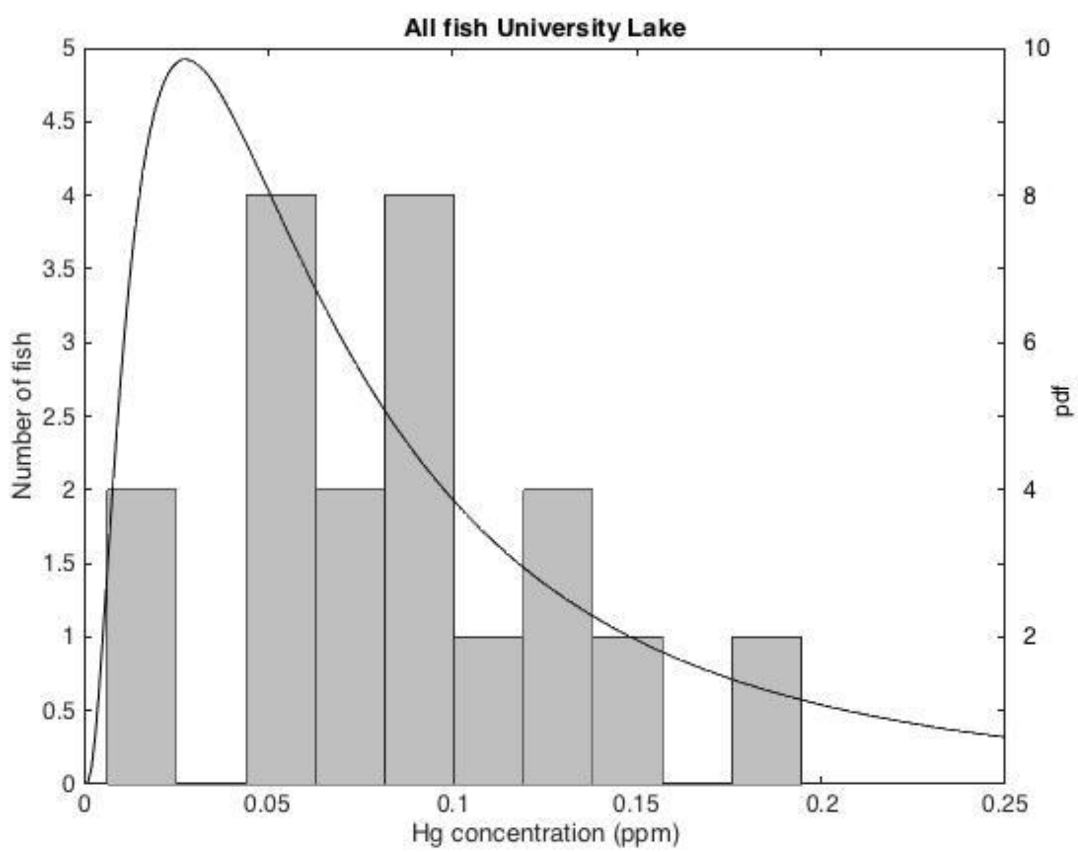

Figure 20. Hg in all fish from University lake

I assumed that the total Hg concentrations in all fish from University Lake were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 20 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (*p*) for the KS test was 0.5647, and I therefore accepted the null hypothesis that the data were log-normally distributed. The probability that Hg would be less than 1.0 ppm was 1.

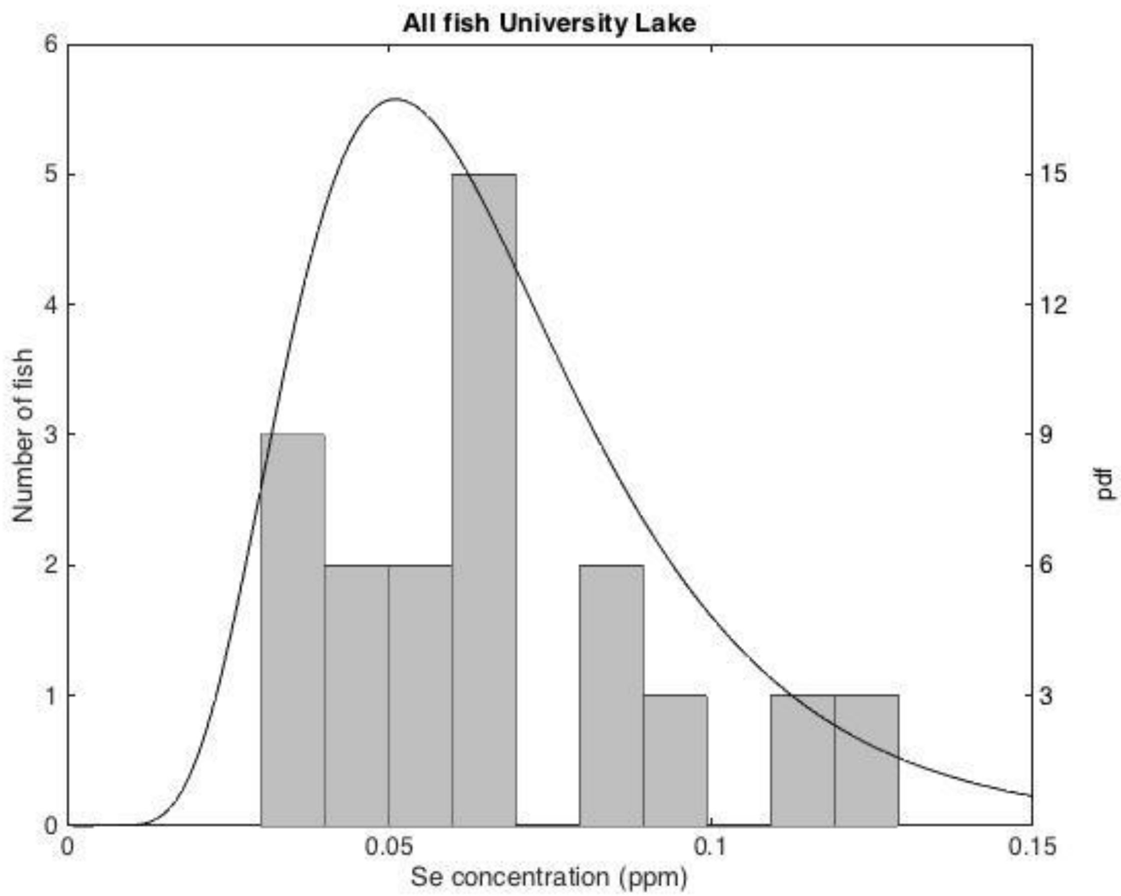

Figure 21. Se in all fish from University lake

I assumed that the total Se concentrations in all fish from University Lake were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 21 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.8909, and I therefore accepted the null hypothesis that the data were log-normally distributed.

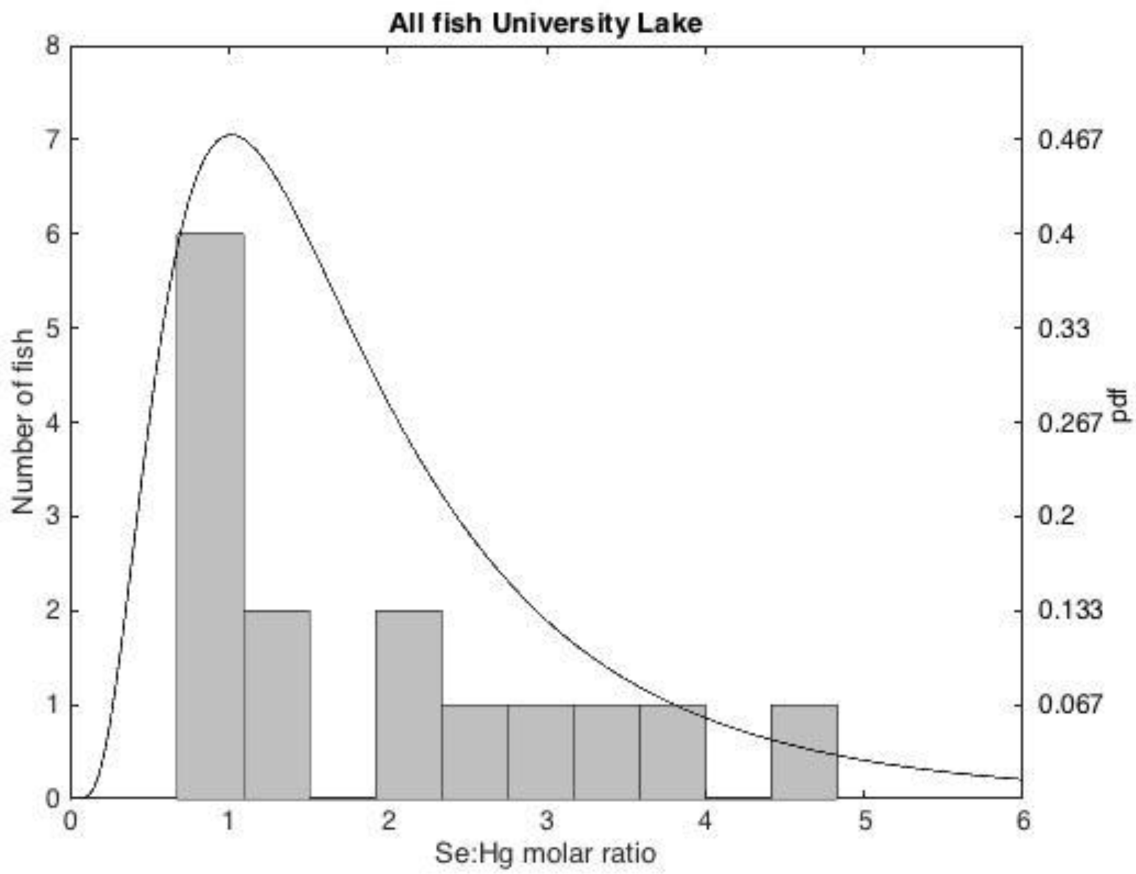

Figure 22. Se:Hg molar ratios in all fish from University lake

I assumed that the Se:Hg ratios for all fish from University Lake were log-normally distributed. I tested the logarithms for normality with a KS test. Figure 22 shows a histogram of the data and the log-normal probability distribution function with the same mean and standard deviation. The type I error rate (p) for the KS test was 0.0015, and I therefore rejected the null hypothesis that the ratios were log-normally distributed. The probability that ratios would be less than 1.0 was 0.4940.

3.4.9. Analysis of Variance for All Sampling Locations

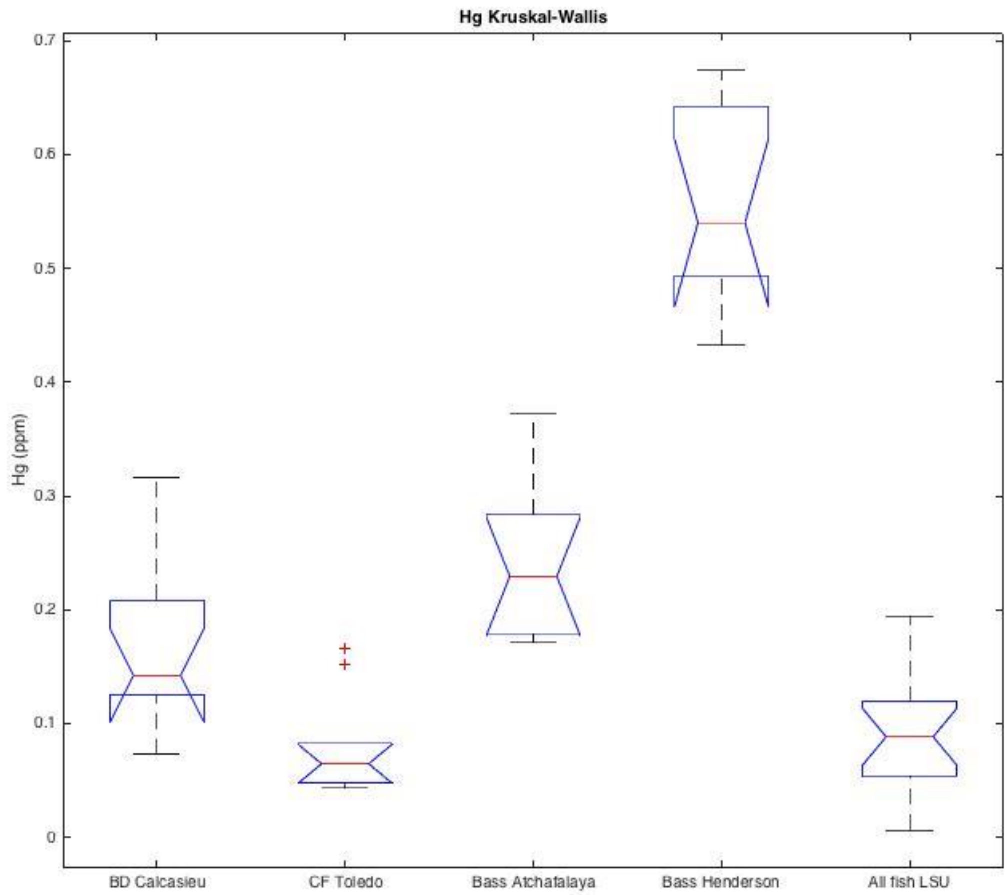

Figure 23. Kruskal-Wallis by locations.

For total Hg, a Kendall test revealed that there were differences in the variances of the bodies of water. Therefore, I used a Kruskal-Wallis test to determine if there were differences in the mean values. The differences in the Hg concentrations between locations were significant ($p < 10^{-7}$). Henderson Lake and the Atchafalaya River had the highest concentrations (geometric mean = 0.36 ppm). Calcasieu Lake was intermediate (geometric mean = 0.15 ppm). Fish from University Lake and Toledo Bend had the lowest Hg concentrations (geometric mean = 0.069 ppm).

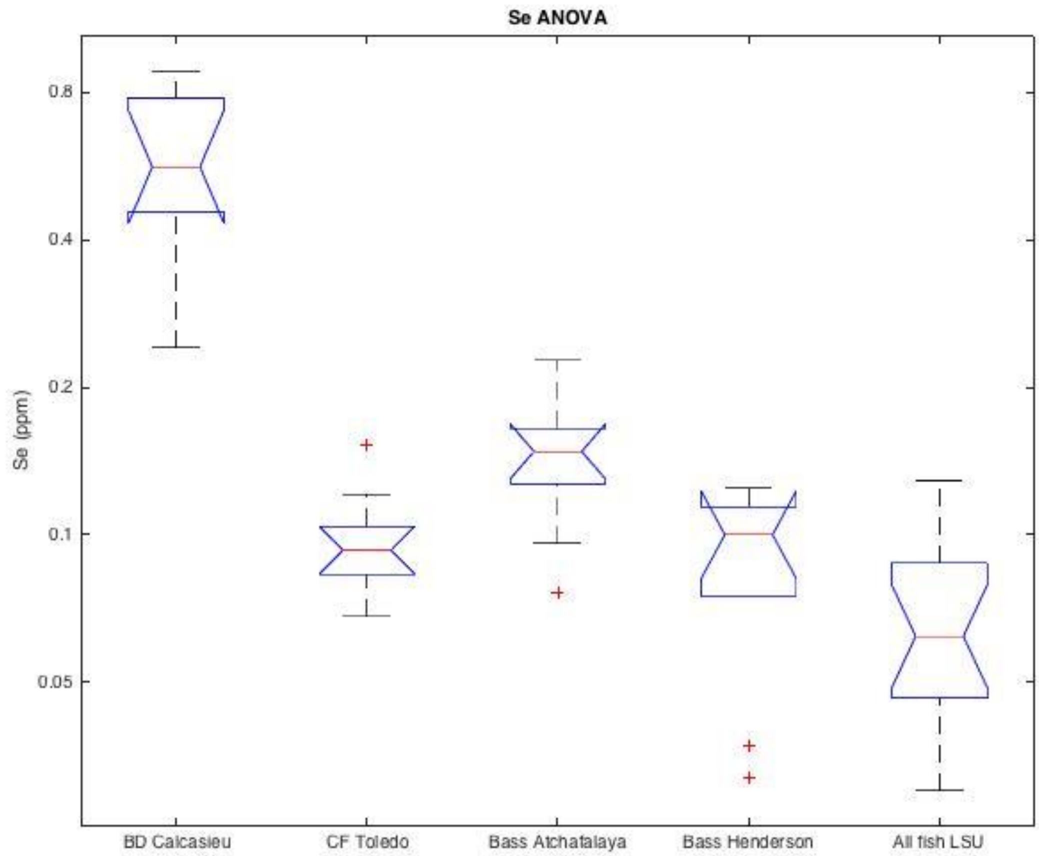

Figure 24. ANOVA for Se by locations.

For Se, The Kendall test revealed no difference in the variances of the log-transformed Se concentrations. I therefore used an ANOVA to determine whether there were differences in the logarithms of the Se concentrations between fish–body-of-water combinations. The type I error rate in this case was 10^{-18}. The highest Se concentrations were in black drum from Lake Calcasieu (geometric mean = 0.55 ppm). The Se concentrations in the fish from the other bodies of water were much lower and not different from one another (geometric mean = 0.086 ppm).

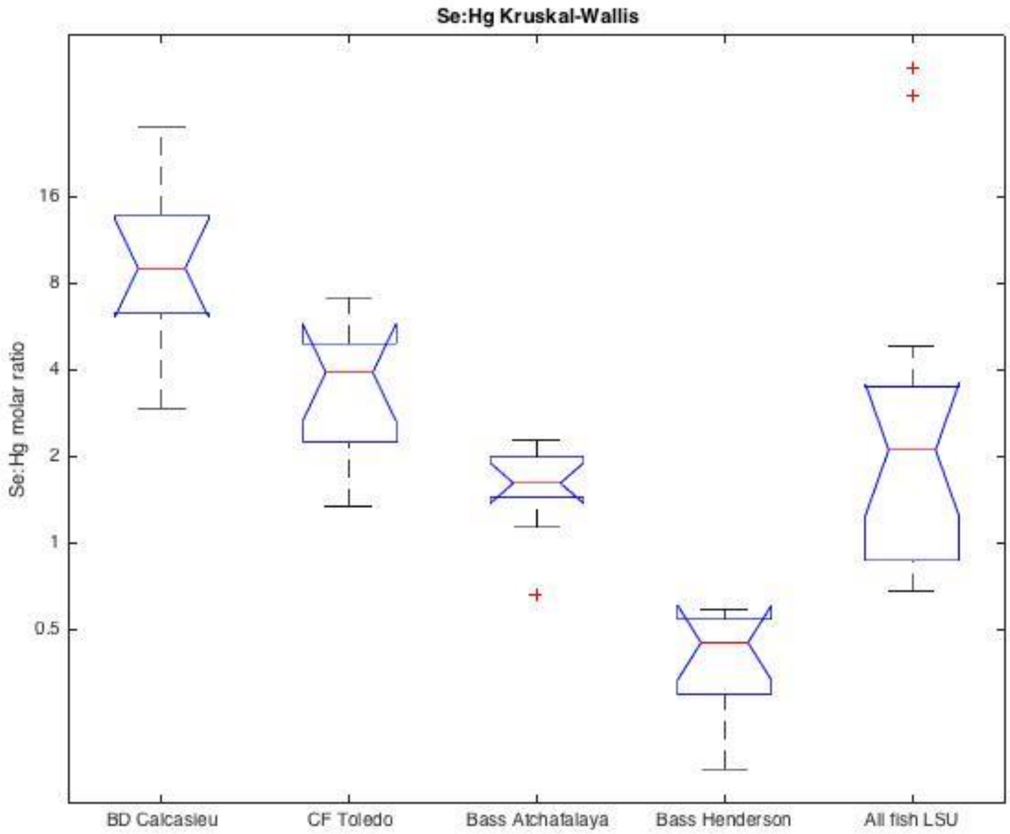

Figure 25. Kruskal-Wallis for Se:Hg by location.

For Se:Hg ratios, I ran a Kendall test first to determine whether there were differences in the variances of the log-transformed data; even after removing the two possible outlier (Gizzard Shad with high Se:Hg ratios), there were still differences in the variances. Then I ran a Kruskal-Wallis test to determine whether there were differences in the ratios between bodies of water. The differences were significant at $p < 10^{-9}$. The ratios were highest in fish from Toledo Bend and Lake Calcasieu (geometric mean = 5.6), intermediate in fish from University Lake and the Atchafalaya River (geometric mean = 1.98), and lowest in fish from Henderson Lake (geometric mean = 0.39).

3.5. Discussion of Results

Black Drum

All commercial samples and duplicates for Black Drum were low in total mercury. The highest concentration was a little over 0.3 ppm, far from the USEPA limit of 1 ppm and the LDEQ limit of 0.88 ppm. Calcasieu Lake is not under a state advisory. The Hg concentrations in this species were a good indication of why Calcasieu Lake is not on the advisory list. The probability that the Hg concentration in black drum is below 1 pm is almost 100%. Overall, Black Drum samples had higher concentrations of Se than total Hg, with a peak at 0.88 ppm, almost triple the highest concentration of total Hg in the same samples. The Black Drum Se-to-Hg molar ratio exceeded 1.0 in 100% of the samples and was as high as 27 in one fish. In general, the Black Drum from Calcasieu Lake were very safe to eat from the standpoint of Hg toxicity. The results of the analysis for methylmercury for all species and locations were not considered in calculating the Se-to-Hg molar ratios because of the apparently poor recovery of MeHg with the analytical procedure that I used. The MeHg concentrations were sometimes several orders of magnitude lower than the total Hg concentrations. However, the results are presented in the Appendix 6.

Catfish

Catfish samples and their duplicates contained the lowest total mercury concentrations. The total Hg concentrations in more than 80% of the samples were below 0.1 ppm, far below the USEPA limit of 1 ppm and the LDEQ limit of 0.88 ppm. However, the state advisories for Hg include Toledo Bend. Catfish samples also contained low concentrations of Se; concentrations in only 3 samples exceeded 0.1 ppm. All samples had Se-to-Hg ratios greater than 1.0 for catfish. Only two ratios were less than 2.0. These samples, in particular, required intense oxidation of the organic matter. These samples were intensely rose-colored, rich in fat and organic matter, perhaps high in omega-3 fatty acids, and other healthy fatty acids. According to customers at the local market, popular knowledge is that catfish are clean fish. The results of this study were in accordance with that urban theory.

Largemouth Bass from the Atchafalaya River

Samples and duplicates of Hg concentrations in largemouth bass were well below the USEPA limit of 1 ppm and the LDEQ limit of 0.88 ppm; the maximum value was 0.37 ppm. Despite the large sizes of the largemouth bass, the Hg concentration was still far from a concern from a human health standpoint. The Atchafalaya River was included in the state advisories for Hg at the time of the sampling, but it was removed in the updated list on February 2018. Se concentrations in largemouth bass samples were variable with a peak at 0.227 ppm. Largemouth bass Se-to-Hg ratios exceeded 1.0 in 10 of 11 fish. However, five ratios were between 1.0 and 1.5. The probability that the ratio was less than 1 based on the log-normal distribution function was 0.15. This result is perhaps a reflection of the fact that bass is a trop-level predator, and the results may reflect biomagnification of Hg. However, from the standpoint of Hg toxicity, the Hg concentrations were still far below 1.0 ppm, and 91% of the Se:Hg ratios exceeded 1. That is in total agreement to its removal from the list of Hg advisories in Louisiana waterbodies in 2018.

Largemouth Bass from Henderson Lake

Largemouth bass specimens from Henderson Lake contained the highest concentrations of total mercury of any fish in the whole study. Four of the 10 fish contained over 0.6 ppm Hg. However, the concentrations were still below the USEPA Hg limit of 1 ppm, albeit closer to the LDEQ limit of 0.88 ppm. The highest total Hg concentration was 0.67 ppm. Henderson Lake is included on the state advisory list. In contrast to the Hg results, the Se concentrations in bass samples from Henderson Lake were very low. The maximum concentration of Se detected was 0.125 ppm, and about 60% of the concentrations were below 0.1 ppm. Hg binds to Se about 1 million times more strongly than it binds to Sulfur. If Hg is highly concentrated in Henderson lake fish, then most of available Se may be sequestered by Hg and may not reach fish tissue for accumulation. Largemouth Bass Se-to-Hg ratios in Henderson Lake specimens were less than 0.58 in 100% of the cases. The probability that those ratios were less than 1 was 0.9661 based on a log-normal distribution function. This is a worst-case scenario from the standpoint of the Se-Hg binding theory. The Hg in these fish therefore represents the greatest threat to human health of the fish included in this study. The USEPA advises that women of childbearing age not consume more than 20/3 = 6.67 micrograms of Hg per day. If the fish contained 0.6 ppm Hg, then consuming more than 11 grams of such fish per day would violate the EPA advisory.

Mercury pollution of Henderson Lake may have resulted from one or both of several postulated mechanisms. The first scenario is that a private company in the plastics-manufacturing business polluted the lake several decades ago. The second scenario states that the oil industry has been responsible for the pollution in the lake as a result of use of Hg in natural gas meters. Presumably they had been in the habit of just throwing the Hg out on the ground when servicing the meters (Al Hindrichs, Louisiana DEQ, personal communication).

Bluegills from University Lake

Bluegill specimens from University Lake were very low in total Hg. About 90% of the samples contained less than 0.1 ppm Hg. The low Hg concentrations are probably related to their small size. The Hg concentrations were very far below the USEPA Hg limit of 1 ppm and the LDEQ limit of 0.88 ppm. University Lake is not listed in the state advisories, and anglers from the local Baton Rouge area are frequently seen fishing near Dalrymple Drive. Bluegill samples were also low in Se; more than 90% of the measurements were below 0.1 ppm Se. The Se-to-Hg molar ratios for bluegill specimens exceeded 1.0 in more than 80% of samples, and the ratios in only two samples fell a little below 1. Nevertheless, the Se:Hg ratios are perhaps insignificant considering that Bluegill samples contain very little Hg. Anglers are probably not be at risk by consuming these fish. Moreover, the small size of the fish may not be appealing for anglers.

Largemouth Bass from University Lake

The Hg concentrations in largemouth bass from University Lake were low; the highest concentration was less than 0.2 ppm. The concentrations were lower than the Hg concentrations in largemouth bass from Henderson Lake and the Atchafalaya River. These Hg concentrations might also be related to the fish size. The largemouth bass from Henderson Lake and the Atchafalaya River were bigger than the LSU largemouth bass. The Se concentrations in largemouth bass from University Lake were also very low; none of the Se concentrations exceeded 0.1 ppm. Given that concentrations of both Hg and Se were low, the conditions around the lake, absence of pollution sources, or natural availability of Se might be the factors to consider as explanations to these low concentrations. The ratios of Se to Hg for largemouth bass in the LSU lake were less than 1.0 in 4 of 4 cases. However, sample 5, which is a duplicate from LSU batch sample 12; might be considered an outlier due to the poor reproducibility on the duplicates for Se analysis via ICP-MS. However, because none of the Hg concentrations exceeded 0.2 ppm, consumption of these fish would not seem to be associated with a risk to human health. In addition, because weight of these fish was no more than 120 g wet weight, it is doubtful that they would be attractive to anglers from a fish consumption standpoint. Larger fish would more likely be consumed. It is worth keeping in mind that the risk to human health is associated with the daily intake of Hg, which means both the concentration in the fish and the quantity of fish consumed matter. For a woman of childbearing age, the daily consumption of fish containing 0.2 ppm Hg should not exceed $20 / (3 \times 0.2) = 33$ grams of fish.

Brown Bullhead Catfish and Gizzard Shad

Two gizzard shad and one of brown bullhead catfish completed the batch from University Lake. According to one local angler resident of Baton Rouge, gizzard shad is not a target species of fishermen. They are too small. Of these three samples of two species, the Hg concentration in the catfish was only 0.12 ppm, despite the fact that the weight of this fish was 345 grams (wet weight). Se concentrations in the gizzard shad were more than 37 times the Hg concentrations, and the Se:Hg molar ratio in the brown bullhead catfish was 1.35. The low concentrations of Hg and Se in brown bullhead catfish are consistent with the low concentrations of both elements in the catfish from Toledo Bend. All of the catfish from Toledo Bend contained more Se than Hg on a molar basis, as did the brown bullhead catfish from University Lake. The implication is that catfish tend to contain low concentrations of both metals, even though there are variations between locations. For gizzard shad, the Se:Hg molar ratios were the highest reported in this study. This would appear to be a species effect because the Se:Hg molar ratios in other fish from University Lake were not especially high.

Variations by Location or Species

At this point it is not possible to say whether location or species has a greater effect on Hg concentrations and Se-to-Hg ratios. To address this issue, I used either one-way analyses of variance (ANOVAs) or Kruskal-Wallis tests to compare species and locations. I used one-way ANOVAs if the data satisfied the assumptions of normality and homoscedasticity (equal variances). If either of these assumptions was violated, I used a Kruskal-Wallis test.

These tests revealed that the total Hg concentrations varied by about an order of magnitude. Concentrations in catfish from Toledo Bend and bluegills from University Lake were similar to each other and low (geometric mean = 0.07 ppm). Hg concentrations were significantly higher in largemouth bass from the Atchafalaya River, black drum from Calcasieu Lake, and largemouth bass from University Lake (geometric mean = 0.18 ppm), and Hg concentrations were significantly higher yet in largemouth bass from Henderson Lake (geometric mean = 0.55 ppm).

The Se concentrations also varied by about an order of magnitude. They were highest in black drum from Calcasieu Lake (geometric mean = 0.55 ppm), intermediate in catfish from Toledo Bend, largemouth bass from Henderson Lake, and largemouth bass from the Atchafalaya River (geometric mean = 0.10), and low in largemouth bass and bluegills from University Lake (geometric mean = 0.057).

Finally, a Kruskal-Wallis analysis of the Se-to-Hg molar ratios revealed that the ratios were low in largemouth bass (geometric mean = 0.77), intermediate in catfish from Toledo Bend and bluegills from University Lake (geometric mean = 2.68), and high in black drum from Calcasieu Lake (geometric mean = 9.15).

Even though, the polluted conditions found in Henderson Lake explained the high Hg concentrations and the theories around it; combined with the relatively clean conditions found in Drum from Calcasieu lake are in total agreement with the location variable prevalence. However, this is not proved for the rest of the sample batches. Analyzing results by species, suggests that Largemouth Bass are top predators and expected to be higher in Hg, but results showed that despite of the dominant Hg content over Se in Henderson and University lake, the ratios where just the opposite for Atchafalaya River. In this case location prevailed. Nonetheless, Catfish, regardless of species and/or location, reported in all cases, low Hg and Se concentrations and Se to Hg ratios higher than 1 in 100% of samples. That apparently suggest a marked species dependence. Though this is true only for catfish, 2 species out of 8 which might not be sufficient to clarify the dominant variable. In any scenario, the variability on the Se content predicted by Ralston in fresh water caught fish was proven (2016). Even though, it is expected to see very low concentrations of Se in fresh water ecosystem compared to rich salty marine ecosystems. According to the USEPA, the acceptable daily intake of mercury is 20 micrograms. For women of childbearing age, that limit is reduced by a factor of 3. When the USEPA set its water quality criteria with respect to Hg, the consumption of fish and shellfish by people in the United States was estimated to average 18.7 grams per day. If the 18.7 grams of fish and shellfish contained 1 ppm Hg, daily intake would be 18.7 micrograms of Hg. This explains why the USEPA action level for mercury in fish is 1 ppm.

However, not everyone consumes 18.7 grams of fish and shellfish per day. That sounds like about one meal of fish per week. Let's suppose that a person consumes two meals of fish per week, something the USEPA recommends that adults do in order to get the full benefits of fish consumption. If that adult happens to be a woman of childbearing age, the concentration of Hg in the fish should not exceed 0.18 ppm. And of course, that assumes that there is negligible intake of Hg from other sources. However, if you are not a woman of childbearing age, that limit goes up by a factor of 3 and becomes 0.53 ppm. The following are the geometric mean concentrations of mercury in the fish that I sampled:

Catfish from Toledo Bend	0.07 ppm
Bluegills from University Lake	0.076 ppm
Largemouth bass from University Lake	0.13 ppm
Black drum from Calcasieu Lake	0.15 ppm

Largemouth bass from the Atchafalaya River	0.24 ppm
Largemouth bass from Henderson Lake	0.55 ppm

A woman of childbearing age could therefore eat two meals per week of Catfish from Toledo Bend, Bluegills from University Lake, Largemouth bass from University Lake, or Black drum from Calcasieu Lake. Furthermore, an adult who is not a woman of childbearing age could eat two meals per week of Largemouth bass from the Atchafalaya River and arguably two meals (certainly one meal) of Largemouth bass from Henderson Lake. By the way, the USEPA (2010 National Listing of Fish Advisories) says that the average concentration of mercury in largemouth bass (17,567 samples) is about 0.52 ppm, which does not make the largemouth bass in Henderson Lake seem all that unusual.

The following list[1] provides an informative comparison of Hg concentrations in fish commonly found in grocery stores and/or served in restaurants:

Tilapia	0.013 ppm
Canned salmon	0.014 ppm
Catfish	0.024 ppm
Freshwater trout	0.071 ppm
Canned light tuna	0.126 ppm
Skipjack tuna	0.144 ppm
Mahi Mahi	0.178 ppm
Canned albacore tuna	0.350 ppm
Bigeye tuna	0.689 ppm

[1] FDA 1990-2012, "National Marine Fisheries Service Survey of Trace Elements in the Fishery Resource" Report 1978, "The Occurrence of Mercury in the Fishery Resources of the Gulf of Mexico" Report 2000

Swordfish	0.995 ppm

Obviously, there are fish being marketed and/or served in restaurants that contain Hg at concentrations comparable to or even higher than the concentrations in the Largemouth bass from the Atchafalaya River and Henderson Lake.

Toxicologists like to remind us that, "The dose makes the poison".[2] This is certainly true in the case of mercury in fish. It appears that an adult male could safely eat one meal per week of Largemouth bass from Henderson Lake without risk of mercury intoxication. With respect to this point, I note that the LDEQ advisory for Henderson Lake says:

> Women of childbearing age and children less than seven years of age should consume no more than ONE MEAL PER MONTH of largemouth bass, crappie, or freshwater drum combined from the advisory area. Other adults and children seven years of age and older should consume no more than FOUR MEALS PER MONTH of largemouth bass, crappie, or freshwater drum combined from the advisory area.

This advice seems very consistent with the results of my study. Ironically, the Se in the fish that contained low concentrations of Hg was probably more than adequate to sequester all the Hg in the fish. Unfortunately, the same could not be said for the Largemouth bass from Henderson Lake.

[2] Adage attributed to the Swiss physician Paracelsus, who actually said, "*Alle Dinge sind Gift, und nichts ist ohne Gift, allein die Dosis macht dass ein Ding kein Gift ist.*
All things are poison, and nothing is without poison, the dosage alone makes it so a thing is not a poison.

Previous Evaluations of Hg in Louisiana

An evaluation of Hg levels in Louisiana fish was published by Katner et al. in 2010. They intended to characterize statewide fish tissue Hg concentrations. Their results showed an overall geometric mean of 0.218 ppm. 95% of their samples had Hg levels below the FDA's action limit of 1.0 ppm. Those reported concentrations are in agreement with my 2017 reported results. 100% of my samples analyzed were below the FDA's action level, even though the differences in sample concentrations were several orders of magnitude. Apparently, the levels of Hg are low in most cases. Species of concern were King mackerel, Blackfin tuna, Largemouth bass, and Freshwater drum (Katner 2010). Form those species, Largemouth bass coincide with my higher Hg concentrations in Henderson Lake. They also reported a small but significant decline in statewide length-adjusted Largemouth bass Hg levels between 1994-1999 and 2003-2008. Aparently, that decline may explain my low Hg concentration levels in some samples of Largemouth bass. They reported a geometric mean for Bass in the range of 0.320 – 0.893. From my results, Henderson lake Hg's geometric mean of 0.55 is in that range.They also reported highest Hg concentration in tissue from Bass, but in general, the majority of samples (>75%) were below the EPA criterion. That was the same for species of black drum, bluegill, and several species of catfish. Most of them were present in my study and reported similar concentration for Hg. Katner et al. (2010) also reported an important variability in Hg by locations when using ANCOVA: Clacasieu, Ponchartrain, Mermentau, Atchafalaya, Sabine, Ouachita, Vermilion-Teche, Terrebonne, Barataria, and Mississippi. The same variability was shown in my study for some of these locations. They located potential hotspot areas that were under advisory. My study suggested the Henderson lake as a hotspot for Hg pollution. At the end they also recommended safe consumption of black drum, channel catfish, and bluegill as my study suggested too.

4. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

4.1. Summary

This study concerned the concentrations of Hg and Se in fish from five different bodies of water in Louisiana: Calcasieu lake, the Toledo Bend reservoir, Atchafalaya River, Henderson Lake, and University lake on the campus of LSU. Se and Hg concentrations were determined in ppm. Se:Hg ratios were calculated on a molar basis. The Hg concentrations and Se-to-Hg molar ratios used to assess the potential threat to human health associated with consumption of fish from these five bodies of water. Differences in Hg, Se, and Se:Hg ratios between species and bodies of water were examined to provide some insight concerning mechanisms and processes.

The first stage of the study consisted of measuring the Hg concentrations in black drum, catfish, largemouth bass from the Atchafalaya River, largemouth bass from Henderson Lake, and bluegill, largemouth bass, brown bullhead catfish, and gizzard shad from University Lake. Results revealed that, with the exception of Henderson Lake, Hg concentrations in these fish never exceeded 0.37 ppm, and in Largemouth bass from Henderson Lake the maximum Hg concentration was 0.67 ppm. All of the Hg concentrations were therefore, below the EPA limit of 1 ppm and the LDEQ limit of 0.88 ppm.

The second stage of the research involved determination of the Se concentrations in the same fish. Se measurements were made with an ICP-MS. The Se concentrations per se were of relatively little interest. The important issue was the molar Se-to-Hg ratios in the fish. With the exception of Largemouth bass, these ratios exceeded 1.0 and were in the approximate range 2–9. Presumably the Se in such fish would sequester the Hg and thereby protect someone who consumed the fish from the toxic effects of the mercury. However, the geometric mean molar Se:Hg in the largemouth bass from University Lake was only 0.80, and the geometric mean

Se:Hg ratio of the largemouth bass in Henderson Lake was only 0.39. Thus, a person who consumed large enough quantities of largemouth bass, particularly from Henderson Lake, might be at risk from the standpoint of mercury intoxication. However, the geometric mean Se:Hg molar ratio in the largemouth bass from the Atchafalaya River was 1.5, presumably high enough to effectively sequester the Hg in the fish.

Therefore, it is not possible to predict which variable (species or location) is dominant to expect higher Se concentrations or lower Hg concentrations by location, or even low concentrations of both by species. The only proved theory is the variability predicted by Ralston (2016) in fish caught from fresh water.

4.2. Conclusions

In general terms, I found that concentrations of Hg in all samples that I analyzed were lower than the USEPA and FDA limit of 1 ppm and the State LDEQ value of 0.88 ppm. They would be a concern to recreational anglers only if the fish were the main course at more than a few meals per week. The ratios of Se to Hg exceeded 1.0 in most of the fish. The only exceptions were the largemouth bass from Henderson Lake and University Lake. Only the former would appear to be a concern from a human health standpoint. The geometric mean Hg concentration in Largemouth bass from University Lake was only 0.13 ppm. The pollution of Henderson lake, whether due to use of mercury in gas meters by the oil and gas industry or use of mercury as a catalyst in the manufacture of plastics (or both) probably accounts in part for the high concentrations of Hg in Largemouth bass from Henderson Lake. However, as noted above, occasional consumption of such fish should not pose a problem from a human health standpoint.

Ralston (2016) has stated: “Se variability is expected in fish caught in freshwaters.” My results are certainly consistent with this statement. The Se concentrations varied by an order of magnitude. However, the Se:Hg molar ratios in the fish consistently exceeded 1.0, with the exception of Largemouth bass from University Lake and Henderson Lake. More extensive sampling and analysis will be needed to clarify this picture, but my work suggests that Largemouth bass probably contain lower Se:Hg ratios than the other species of fish that I sampled.

4.3. Recommendations

Given the variability of Se concentrations between locations and species; further studies of the concentrations of Se in freshwater fish in Louisiana seems warranted. The issue of concern is whether current state advisories are too strict. The advisories at present include the Toledo Bend Reservoir and the Calcasieu River drainage basin. Adults, for example, are advised not to eat more than four meals per month of freshwater drum from the Calcasieu River drainage basin. The results of this study suggest that fish from those bodies of water do not contain high concentrations of Hg and my well contain enough Se to effectively sequester whatever Hg is in the fish.

The results of this study suggest a positive scenario for recreational anglers. In short, it does not appear difficult for recreational anglers to keep their Hg intake below the recommended EPA thresholds of 6.67 µg of methylmercury per day for women of childbearing age and 20 µg of methylmercury per day for other adults. However, this EPA assumption is made by fish consumption on a daily basis and from the same body of water (Laws 2018). The LDEQ limit of 0.88 ppm of Hg in fish is based on the assumption that people in Louisiana eat an average of 24 ounces of fish per month = 22.68 grams per day. The limit of 0.88 ppm is therefore consistent with the FDA recommendation that the daily intake of Hg not exceed 20 micrograms. This limit is reduced by a factor of 3 for women who are pregnant or nursing. The action level in fish would therefore become 0.29 ppm on a fresh weight basis. However, it seems unlikely that people would eat the same fish, from the same body of water, day after day. There are certainly plenty of fishing holes in Louisiana. My recommendation would therefore be that Cajun fish fans should include a variety of fish in their diet and, if they want to eat fish every day, frequently change the place where they fish.

REFERENCES

Anestål, Karin, and Elias S J Arnér. 2003. "Rapid induction of cell death by selenium-compromised thioredoxin reductase 1 but not by the fully active enzyme containing selenocysteine." *The Journal Of Biological Chemistry* 278 (18): 15966-15972. Accessed December 21, 2017. https://eds-b-ebscohost-com.libezp.lib.lsu.edu/eds/detail/detail?vid=10&sid=ad3ba33d-38e4-449c-a81f-a9bced5a0c56%40sessionmgr120&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=12574159&db=cmedm.

Branco, Vasco, João Canário, Arne Holmgren, and Cristina Carvalho. 2011. "Inhibition of the thioredoxin system in the brain and liver of zebra-seabreams exposed to waterborne methylmercury." *Toxicology And Applied Pharmacology* 251 (2): 95-103. Accessed Diciembre 21, 2017. doi:10.1016/j.taap.2010.12.005.

Carbonell, G, J C Bravo, C Fernández, and J V Tarazona. 2009. "A new method for total mercury and methyl mercury analysis in muscle of seawater fish." *Bulletin Of Environmental Contamination And Toxicology* (Springer Science+Business Media, LLC 2009) 83 (2): 210-213. Accessed December 30, 2017. doi:10.1007/s00128-009-9720-x.

CDC-ATSDR. 2017. *ATSDR, the Agency for Toxic Substances and Disease Registry: Toxic Substances Portal.* October 2. Accessed December 22, 2017. https://pubchem.ncbi.nlm.nih.gov/source/CDC-ATSDR%20Toxic%20Substances%20Portal.

Ceccatelli, Sandra & Aschner, Michael. 2012. *Methylmercury - Toxicology - Neurotoxicology.* New York: Springer. Accessed December 20, 2017. http://libezp.lib.lsu.edu/login?url=http://dx.doi.org/10.1007/978-1-4614-2383-6 .

CEM Corporation. 2001. "MARS - Microwave Digestion System." *CEM.* April. Accessed December 30, 2017. http://cem.com/en/mars-6/.

Coyne, Mark S. 2013. *Selenium (Se).* Ipswich, MA: Salem Press Encyclopedia Of ScienceResearch Starters. Accessed December 22, 2017. https://eds-b-ebscohost-com.libezp.lib.lsu.edu/eds/detail/detail?vid=2&sid=afbd0443-6a6f-45ad-9b29-f299edfca909%40pdc-v-sessmgr01&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=89474872&db=ers.

CRC John R. Rumble. 2017-2018. *Handbook of Chemistry and Physics.* 98. Edited by CRC Press. Prod. John R. Rumble. U.K., December 22. Accessed December 22, 2017. http://hbcponline.com/faces/contents/ContentsSearch.xhtml.

Crosby, Donald G. 1998. *Environmental Toxicology and Chemistry.* Oxford: Oxford University Press Inc.

DeLaune, R D, A Jugsujinda, I Devai, and W H, Jr Patrick. 2004. 2004. "Relationship of sediment redox conditions to methyl mercury in surface sediment of Louisiana Lakes." *Journal Of Environmental Science And Health. Part A, Toxic/Hazardous Substances & Environmental Engineering* 39 (8): 1925-1933. Accessed December 20, 2017. https://eds-b-ebscohost-com.libezp.lib.lsu.edu/eds/detail/detail?vid=5&sid=615fd449-b5f2-4de9-bcb571b8a79d5de1%40sessionmgr101&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=15332659&db=cmedm.

EPA. 2010. "Environmental Protection Agency." *Environmental Protection Agency.* Accessed April 11, 2017.

—. 2010. "Environmental Protection Agency." *Environmental Protection Agency.* April. Accessed April 11, 2017. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1007BKQ.PDF?Dockey=P1007BKQ.PDF.

—. 2017. *United States Environmental Protection Agency.* December 22. Accessed December 28, 2017. https://www.epa.gov/fish-tech/2017-epa-fda-advice-about-eating-fish-and-shellfish.

Felix Beckers, Jörg Rinklebe. 2017. "Cycling of mercury in the environment: Sources, fate, and human health implications: A review." Edited by LLC Taylor & Francis group. *Critical Reviews in Environmental Science and Technology* (Taylor & Francis group, LLC) 49 (9): 1-102. Accessed 12 17, 2017. https://eds-b-ebscohost-com.libezp.lib.lsu.edu/eds/detail/detail?vid=6&sid=dc891daf-64ed-41dc-a6d7-56660e63e228%40sessionmgr120&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=125941405&db=a9h.

Gates, Keith W. 2016. "Seafood Science: Advances in Chemistry, Technology and Applications." Edited by Se-Kwon Kim. *Journal Of Aquatic Food Product Technology* (CRC Press Tyalor and Francis group) 25 (2): 281-285. Accessed December 25, 2017. doi:10.1080/10498850.2016.1129822.

Gutierrez, Ben. 2012. "Hawaii News Now." *Hawaii News Now.* October 07. Accessed April 11, 2017. http://www.hawaiinewsnow.com/story/19759433/new-uh-study-okay-for-pregnant-women-to-eat-most-fish.

Hardy, Steve. 2017. "Testing resumes of mercury levels in La. fish." *Metro,* March 12: 4B.

Hatfield, Dolph L., Marla J. Berry, and Vadim N. Gladyshev. 2012. *Selenium. [electronic resource] : its molecular biology and role in human health.* New York, NY, NY: Springer. Accessed December 22, 2017. https://eds-b-ebscohost-com.libezp.lib.lsu.edu/eds/detail/detail?vid=0&sid=4a3cc2fd-a4a3-4431-9cd3-

4a6a8923b7cb%40pdc-v-sessmgr01&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=lalu.4055608&db=cat00252a.

HSDB. 2017. *Selenium Elemental.* Specialized Information Services. Bethesda, MD, December 19. Accessed December 22, 2017. http://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm.

ILO-ICSC. 2017. *The International Chemical Safety Cards (ICSC) / International Labour Organization.* December 2. Accessed December 22, 2017. https://pubchem.ncbi.nlm.nih.gov/source/ILO-ICSC.

JÖrg Rinklebe, Anna Sophia Knox, Michael Paller. 2017. *Trace Elementsin Waterlogged Soils and Sediments.* Boca Raton, FL: CRC Press Tyalor & Francis Group.

Kaneko, J John, and Nicholas V C Ralston. 2007. "Selenium and mercury in pelagic fish in the central north pacific near Hawaii." *Biological Trace Element Research* (Humana Press Inc.) 119 (3): 242-254. Accessed December 26, 2017. doi:0.1007/s12011-007-8004-8.

Katner, Adrienne, Mei-Hung Sun, Mel Suffet. 2010. "An evaluation of mercury levels in Louisiana fish: Trends and public health issues." *Science of the Total Environment* (Elsevier B.V.) 408: 5707-5714. doi:10.1016.

Korbas, M., O'Donoghue, J. L., Watson, G. E., Pickering, I. J., Singh, S. P., Myers, G. J., & ... George, G. N. 2010. "The chemical nature of mercury in human brain following poisoning or environmental exposure." *ACS Chemical Neuroscience* 1 (12): 810-818. Accessed December 21, 2017. doi:10.1021/cn1000765.

Lands, William E.M. 1986. *Fish and Human Health.* Chicago, IL, Ilinois: Academic Press, Inc. Accessed March 11, 2018.

Laws, Edward. 2018. *Aquatic Pollution: An Introductory Text.* 4. John Wiley & Sons Ltd.

Lincoln, Rebecca James P. Shine, Edward J. Chesney, Donna J. Vorhees, Philippe Grandjean, and David B. Senn. 2011. "Fish Consumption and Mercury Exposure among Louisiana Recreational Anglers." *Environmental Health Perspectives* 2: 245. Accessed December 29, 2017. doi:10.1289/ehp.1002609.

Louisiana Department of Environmental Quality. 2003. *Resource Guide to Understanding Mercury in Louisiana's Environment.* Annual Mercury Report, LDEQ, Baton Rouge: Louisiana Department of Environmental Quality. Accessed December 27, 2017.

Louisiana Department of Health and Hospitals. 2012. *PROTOCOL FOR ISSUING PUBLIC HEALTH ADVISORIES FOR CHEMICAL CONTAMINANTS IN RECREATIONALLY CAUGHT FISH AND SHELLFISH.* Protocol, Office of Public Health, Section of Environmental Epidemiology and Toxicology (SEET), Baton Rouge: Section of Environmental Epidemiology and Toxicology (SEET) LDHH. Accessed December 28, 2017. http://www.dhh.louisiana.gov/offices/page.asp?id=205&detail=5749.

Medical Subject Headings. 2017. *US National Library of Medicine.* National Library of Medicine, Maryland, December 20. Accessed December 22, 2017. http://www.ncbi.nlm.nih.gov/mesh.

Milestone Srl. 2013. *Milestone Helping Chemists, DMA-80.* Accessed December 30, 2017. https://www.milestonesrl.com/en/mercury/dma-80/features.html.

National Center for Biotechnology Information. 2004. "PubChem Chemistry Compound Data Base." *NIH National Institute of Health: US National Library of Medicine,* . Bethesda, MD, MD, 09 16. Accessed 12 16, 2017. https://pubchem.ncbi.nlm.nih.gov/compound/23931#section=Top.

NCIt National Cancer Institute. 2017. *Selenium.* Rockville, MD: U.S. National Library of Medicine. Accessed December 22, 2017. https://pubchem.ncbi.nlm.nih.gov/source/NCIt.

Nelms, Simon. 2016. *Analyte Guru.* Febrero 19. Accessed December 31, 2017. http://analyteguru.com/the-dark-side-of-the-moon-selenium-analysis-using-icp-ms-part-2/.

OSHA. 2017. *OSHA Chemical Sampling Information.* November 11. Accessed December 22, 2017. https://www.osha.gov/dts/chemicalsampling/toc/toc_chemsamp.html.

Poulain A.J., Tamar Barkay. 2013. "Cracking the Mercury Methylation Code." *Science* 339 (6125): 1280-1281. Accessed December 20, 2017. doi:10.1126/science.1235591.

Ralston, Nicholas & J. Raymond. 2016. "The “SOS” Mechanisms of Methylmercury Toxicity." *Global Advances in Selenium Research from Theory to Application* (Taylor & Francis Group, London ISBN 978-1-138-02731-2) 1-2. Accessed November 2017.

Ralston, Nicholas V.C., Laura J. Raymond. 2010. "Dietary selenium’s protective effects against methylmercury toxicity." *ELSevier* 112-123.

Science is Fun. 2017. *Mercury.* University of Wisconsin-Madison, Wisconsin, 01 01. Accessed December 20, 2017. http://www.scifun.org/CHEMWEEK/Mercury2017.pdf.

Stringari, James, Adriana K C Nunes, Jeferson L Franco, Denise Bohrer, Solange C Garcia, Alcir L Dafre, and Marcelo Farina, et al. 2008. "Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain." *Toxicology And Applied Pharmacology* 227 (1): 147-154. Accessed December 21, 2017. https://eds-b-ebscohost-com.libezp.lib.lsu.edu/eds/detail/detail?vid=2&sid=ad3ba33d-38e4-449c-a81f-a9bced5a0c56%40sessionmgr120&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=18023834&db=cmedm.

US EPA. 1996. "SW-846 Test Method 3052." *United States Envionmental Protection Agency*. December. Accessed January 1, 2018. https://www.epa.gov/hw-sw846/sw-846-test-method-3052-microwave-assisted-acid-digestion-siliceous-and-organically-based.

APPENDIX A. REVIEW OF LITERATURE: INTRODUCTION TO METALS

A.1 Metals in the Environment

The potentially devastating effects that heavy metals can have in human health have made metal pollution a significant point of aquatic pollution research. Lead and Arsenic in tap water and the incident of mercury pollution in Minamata, Japan, are a few major examples of heavy metal pollution (Laws 2018). The adverse effects of metals in humans have been recorded since ancient times. In most cases, polluted water was the vehicle by which metals reached humans, either by drinking water or consumption of contaminated fish or shellfish. Metals reach aquatic ecosystems by several processes, including: weathering of soils and rocks, volcanic eruptions, and anthropogenic activities involving mining, processing, or use of metals and derivates (Laws 2018).

Some metals such as: iron, copper, and zinc, are essential micronutrients. However, some others, including mercury and lead, are not required by any organism at any level. Both groups of metals are toxic to aquatic organisms and humans when levels of exposure are high enough (Laws 2018). The metals of concern are in most cases heavy metals—metals with relatively high densities, atomic weights, or atomic numbers.[3] They are potentially toxic when present in soils, wetlands, sediments, and water bodies. Sediments and wetland soils have special properties that may impact metal distribution, mobility, reactivity, and toxicity. Toxic trace-metals can be found in different species in sediments, wetland soils and surface waters (Rinklebe et al. 2017). Metallic species readily available to aquatic, benthic organisms, and plants include: metals dissolved in soils, surfaces, interstitial waters, and those bound to the solid phase by cation ex-

[3] So-called light metals include magnesium, aluminum, and titanium.

change processes. That means: pH and redox conditions are the two most important factors that determines metal's mobility (Rinklebe et al. 2017).

The term "heavy metals" include elements such as cadmium, cooper, zinc, and nickel. According to Crosby (1998) the authentic heavy metals are: mercury, thallium, lead, and bismuth. Their atomic weights range between 200 and 210. They have a characteristic conductivity, appearance and tend to form covalent compounds. They are very toxic and share some toxic characteristics similar to those of arsenic (Crosby 1998).

The majority of metals are insoluble in water with a neutral or basic pH. They are absorbed to particulate matter or bioaccumulated in living organisms. The availability of metals plays a central role on determining toxicity. However, some processes can contribute to the immobilization of metals: (1) transformation to oxides, hydroxides, and carbonates of low solubility; (2) capture or absorption to colloidal hydrous oxides of iron and manganese under aerobic, neutral, or alkaline pH; (3) precipitation to highly insoluble sulfides under reducing conditions; and (4) complexation with humic materials (Rinklebe et al. 2017).

Thus, Metals may undergo transformations between active and inactive species that affect their mobility and availability mainly because of changes in the physicochemical properties of the system: pH, redox potential, and salinity (Rinklebe et al. 2017). Metals associated with particulate matter are unlikely to exert toxic effects on aquatic organisms but, it is possible that metals could be desorbed in acidic environments or be absorbed by an organism in the process of pumping water over its gills (Laws 2018).

A.2. Mechanism of General Toxicity

The high toxicity of arsenic (As) and other metals is related to the stability of its bonds with sulphur. For example, in cellular process, the pyruvate residue of glycolysis is transformed to the metabolic building block: acetyl coenzyme A by the enzyme pyruvate dehydrogenase (PDH). Then, Pyruvate condenses with thiamine pyrophosphate (TPP) to produce Hydroxyethyl-TPP, which acetylates dihydrolipoyl transacetylase (DLT). DLT, then transform coenzyme A into Acetyl Co-A. the active site of enzyme is the 1-3 dithiol, dihydrolipoamide DHL. There, As^{3+} bonds covalently. The driving force of the reaction is big because the As-S bond angles allow formation of ring (Crosby 1998).

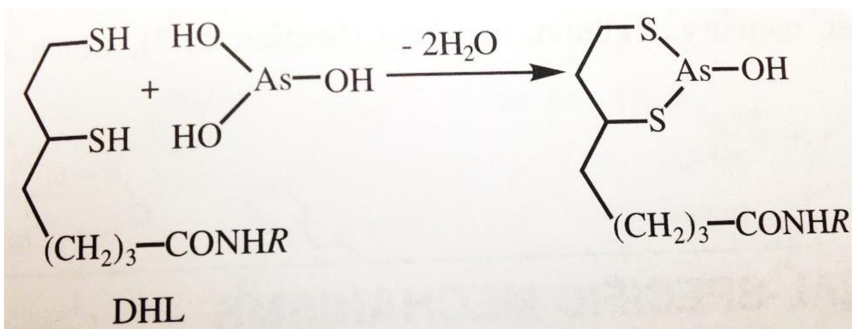

Figure A.1. binding of As^{3+}, source: (Crosby 1998).

The Enzyme DHL mediate the transfer of electrons in the same way as the activated acyl groups generated by the oxidation of glucose. The deactivation of DHL by As results in the loss of the energy derived from glycolysis, which is the source of energy for cells. Respiration is therefore, inhibited and then cells from humans, animals, plants, and even microorganisms die because of progressive respiratory inactivity (Crosby 1998).

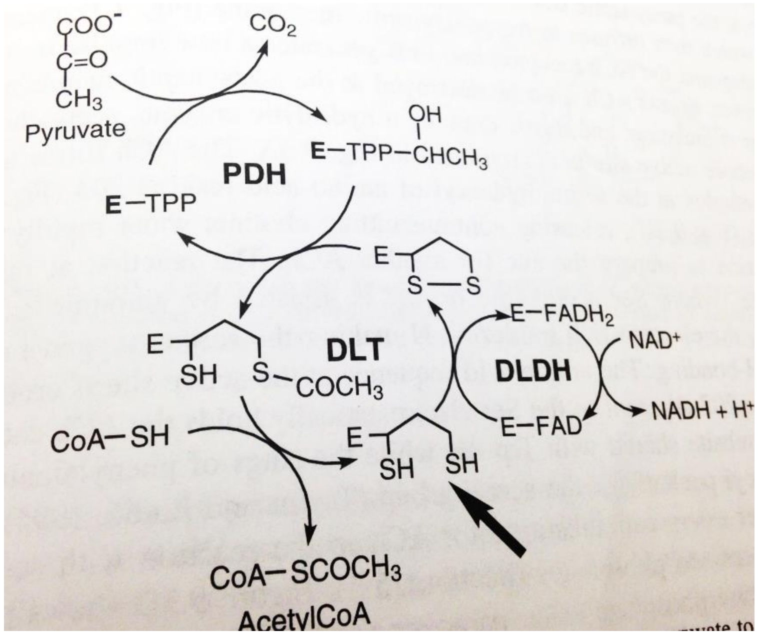

Figure A.2. Inactivation of DHL enzyme by As^{3+} (Crosby 1998).

Certain metals such as mercury, lead and cadmium exert toxic effects due to their tendency to combine with sulphur-containing amino acids in proteins. This produce interference with enzyme-mediated process or disruption of cellular structure (Crosby 1998).

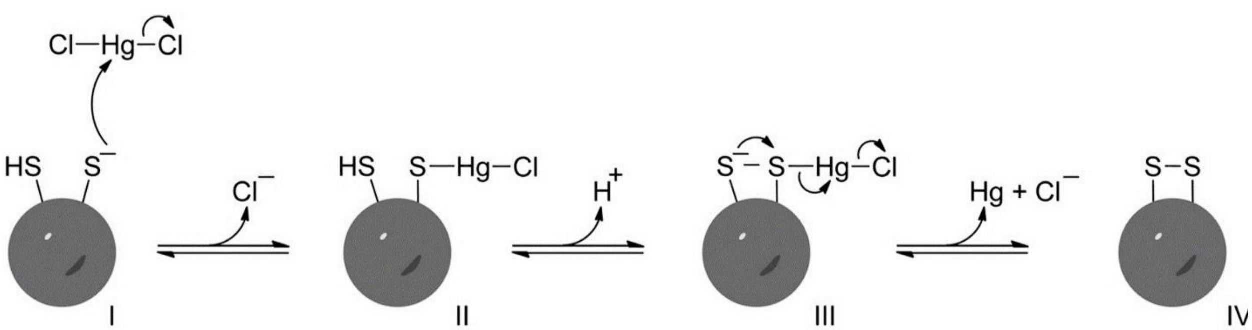

Figure A.3. Oxidation by $HgCl_2$ of two closely spaced cysteine sulfur atoms to form a disulfide bond. source: Biochemical Journal, Portland Press, August 2011.

http://www.biochemj.org/content/437/3/455

APPENDIX B. MERCURY LITERATURE REVIEW

B.1. General Properties

Mercury(Hg) is a toxic heavy metal with several chemical names: “Hydrargyrum, Quicksilver, Metallic mercury, Liquid silver.” (National Center for Biotechnology Information 2004). Hg’s name comes from Roman god Mercury. Its chemical symbol, Hg, derives from its Latin name, hydrargyrum, which means liquid silver.” (Science is Fun 2017) Several minerals of Hg are known but, the most abundant Hg compound is HgS, which can be found in three polymorphs: cinnabar (which represents most of the mercury extracted), meta-cinnabar, and very rarely in hyper-cinnabar (Beckers and Rinklebe 2017).Table 7 summarizes general properties of Hg:

Table B.1. Properties of Hg

Atomic number Atomic weight	80 200.5924 g/mol
Melting point	–38.8 °C
Boiling point	356.7°C
Density	13.534 g/cm^3
Specific gravity	13.55
Vapor pressure	1.22×10^{-3} mm Hg at 20°C 2.8×10^{-3} mm Hg at 30°C
Aqueous solubility	5.6×10^{-7} g/L at 25°C

Source: (Beckers and Rinklebe, 2017).

Description of Hg as a silver metallic element, found as liquid at room temperature, odorless, insoluble in water, dilute hydrochloric acid, hydrogen bromide, hydrogen iodide, cold sulphuric acid but it is soluble in nitric acid and to some extent in lipids, even pentane (National Center for Biotechnology Information 2004). Physical properties of Hg reveals poor conduction of heat and a fair conductor of electricity, but enhanced by its coefficient to thermal expansion for use in electrical devices. Table 8 summarizes historical applications:

Table B.2.. Historical uses of mercury

Catalyst in chlor-alkali production
Fungicide in paints and on seed coatings
Scientific instruments
Anti-fouling paint
Control devices
Mirror coatings
Medical devices
Dental fillings

Source: (National Center for Biotechnology Information 2004)

Hg, is toxic if ingested, absorbed, or inhaled in the form of mercury vapor. Absorption through the skin and mucous membranes results in Hg poisoning. (National Center for Biotechnology Information 2004). Thus, Hg is a non-essential trace metal, and is widely recognized as toxin. Usage of mercury has been phased out in the United States, except for the amalgams used in dental fillings. Toxicity of Hg is only second to lead in heavy metal poisoning. (National Center for Biotechnology Information 2004).

B.2. Biochemistry and Physiology

Most of Hg's toxicity comes from inhalation of vapors causing irritation of eyes and skin. Absorption in the skin is slow, however, ingestion of Hg is not a significant route of acute exposure because it's poorly absorbed in the stomach (NIOSH 2017). Many studies have revealed a correlation between the number of dental amalgam fillings in humans, and the Hg content in brain and kidney from human autopsies. (National Center for Biotechnology Information 2004)

Target organs include: developmental brain in child, neurons in adults, gastrointestinal, nervous system, ocular and renal. Toxicity of Hg vapor comes from the divalent mercury produced by the oxidation occurring on the brain tissue. NIOSH suggested potential DNA damage (2017). MeHg is classified as possibly carcinogenic to humans (not evaluated yet) (National Center for Biotechnology Information 2004). Acute symptoms for Hg vapor contact are tremors, irritability, insomnia, memory loss, neuromuscular imbalance, headaches, slowed sensory and motor nerve function. (National Center for Biotechnology Information 2004). In Chronic exposure symptoms are manifested in Nervous system effects such as erethism (increased excitability), irritability, excessive shyness, insomnia, severe salivation, gingivitis, and tremors. Excretion may include kidney damage, manifested by proteinuria. The biological half-life of Hg; in fish is around 2 to 3 years, but in the whole body of a human has a value of 50 to 70 days. (National Center for Biotechnology Information 2004).

B.3. Uses by Chemical species:

Table 9 summarizes current and old appliances of Hg that exposes humans to it. In the U.S., Dental amalgams are still being used:

Table B.3. Uses of Hg by chemical species. Source: (Beckers & Rinklebe 2017) and (NCBI 2004).

Elemental Hg	**Inorganic Hg**	**Organic Hg**
Dental fillings (amalgams)	Hg_2Cl_2 used as disinfectant, fertilizers, and pesticide.	Methylmercury has no appliances.
Manufacturing of thermometers, and barometers	Laxative, skin lightening creams and soaps	
Batteries, lamps, fluorescent light bulbs.	Latex paint.	
Industrial processes, refining, lubrication oils.	Antisyphilitics, astringents.	
Electrical devices: switches and control equipment.		
Chlor-alkali industry and mining.		

B.4. Mercury in the Environment

The long Hg's atmospheric lifetime and the contribution of anthropogenic emissions together account for the long-dwelling period of Hg in the atmosphere. Hg vapor is more than 95% of the mercury found in the atmosphere. However, mercury in water, sediments, and soils is found in the inorganic form Hg (II) but, mono-methylmercury (CH_3Hg^+) is the dominant species in biological systems. Emissions of Hg have varied sources, even natural but, coal-fired power plants and incineration of some medical devices are major anthropogenic contributors. (Beckers & Rinklebe 2017).

B.4.1. Naturally occurring mercury

Mercury is present in all types of rocks. The upper crust of the Earth encompasses around 0.05 ppm of Hg. Lower Earth crust layers are approximately from 0.014 ppm to 0.0079 ppm (Beckers et al. 2017). Hg's affinity for organic materials lead it to concentrate in black shales, coal, petroleum, and natural gas deposits. (Beckers et al. 2017). Geochemical processes such as hydorthermal reworking of marine black shales can also contribute to the concentration and precipitation of minerals containing Hg such as those found in volcanic regions.(Rinklebe et al. 2017)

B.5. Effects on the Environment

B.5.1. Effects in Aquatic environments

The main issue of Hg in water ecosystems comes from toxicity and bioaccumulation/biomagnification of MeHg in biological systems, reaching the foodchain, and ultimately humans. Uncommon mortality, growth and behavior disturbance, reproduction, and reproductive impairment, neurotoxic and embryotoxic effects in several fish species were reported (Beckers et al. 2017).

B.5.2. Effects on Terrestrial Ecosystems

MeHg in the aquatic food-chain may eventually reach terrestrial predators which feed on coastal zones, increasing the pollution extent. (Beckers et al. 2017).

B.5.3. Exposure of Humans

The fish and Shellfish comsumption is typically the main pathway for MeHg into the human body. However, frequent ingestion of polluted rice meals is another significant source of MeHg in some regions of the world (Beckers 2017).

APPENDIX C. METHYL MERCURY LITERATURE REVIEW

C.1. Methylation and Risks

As Hg cycles in the environment, a series of complex physical and chemical reactions produce organic Hg (EPA 2010). Three major groups of mercurial organics include: Phenyl Hg (i.e. phenyl mercuric acetate or PMA), methoxy Hg (i.e. methoxyethyl mercury acetate), and alkyl Hg (i.e. methylmercury, MeHg) (Laws 2018). MeHg is commonly produced by microbial activity in wetlands, sediments and water by a reaction called methylation (EPA 2010). MeHg's risk to human health lies on its ability to pass the human blood-brain barrier. MeHg is a potent neurotoxin able to cause severe and irreversible damage to adults but more severely in children (Laws 2018).

C.1.2. The Bacterial Methylation

Hg's atmospheric deposition is taken up by bacteria, initiating the methylation process. This process transfers a methyl group (--CH_3) to inorganic Hg^{2+}. Methylation is made with methyl cobalamin, a vitamin 12 analogue that can be produced by enzymatic reactions or by electrophilic attack of Hg^{2+} to methyl-cyano-cobalamin (Laws 2018).

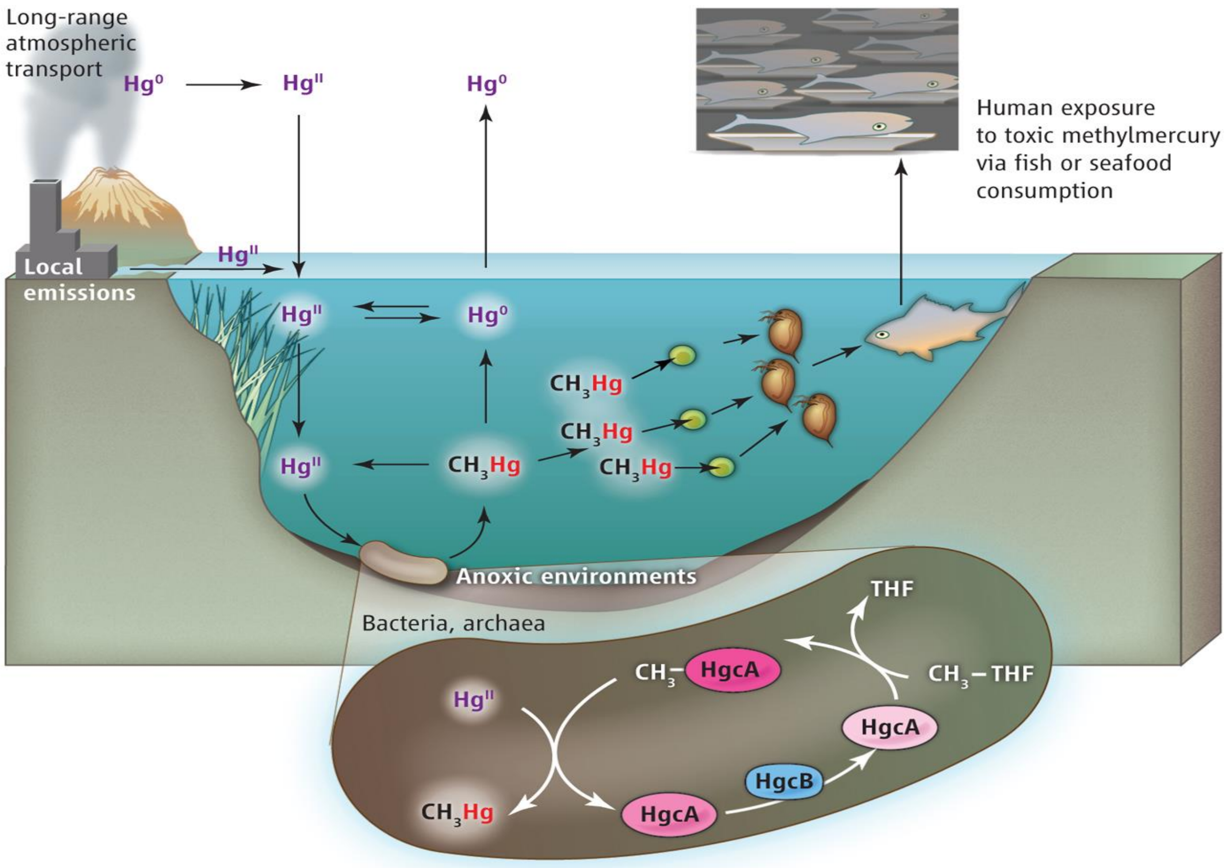

Figure C.1. Methylation of Hg. (Poulain 2013).

Methylation can take place under aerobic and anaerobic conditions. However, in case of anaerobic conditions, the formation of mercury sulfide might prevent the reaction (Laws 2018). MeHg is more lipophilic and reactive, after its cellular absorption, it is conserved and bioaccumulated. Accumulation starts in bacteria/bacterioplankton and phytoplankton; these are consumed in the next trophic level. Thus, bioaccumulation continues to higher tropic levels in the food web. Biomagnification results from the accumulation of higher Hg concentrations in top predators of the food web. Both processes can occur in marine and freshwater food webs (Laws 2018).

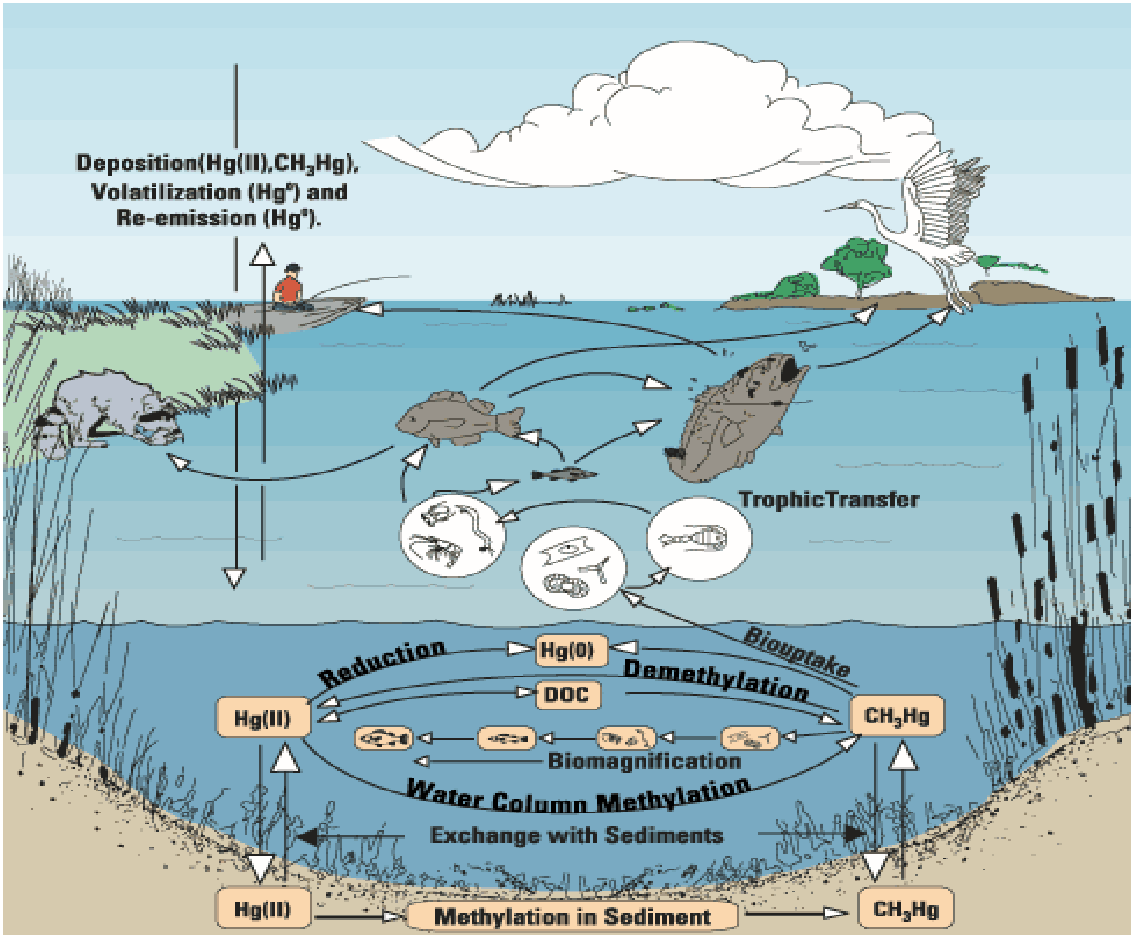

Figure C.2. Biomagnification of MeHg. (Science is Fun 2017).
http://scifun.chem.wisc.edu/chemweek/mercury/mercury.htm

C.2. Factors influencing Methylation

Several factors govern Hg methylation and uptake in shellfish and fish. Hg^{2+} is predominant in soils, water and sediments, part of it, is converted to MeHg by microbial reactions (EPA 2010). Methylation or demethylation rates are influenced by redox potential, pH, sulfate content, and microbial activity. Methylation in sediments is conducted by anaerobic sulfate reducing bacteria such as Desulfovibrio, favored by reducing conditions (Rinklebe et al. 2017). High salinity levels (sulfates) inhibit methylation. Organic matter in sediments stimulate methylation but, under all conditions, sulfate-reducing bacteria are the key participants (DeLaune 2004). Numerous pathways lead to demethylation of MeHg. However, the dominant process is oxidative demethylation in aerobic sediments (Rinklebe et al. 2017). In water, MeHg is also degraded by sunlight (EPA 2010).

C.3. Factors influencing Bioaccumulation

Bioaccumulation is present through each successive trophic level starting from benthic and pelagic levels. MeHg is almost exclusively found in predatory freshwater fish. Bioaccumulation is a function of several uptake (diet and gills) and excretion pathways. Factors affecting bioaccumulation include: pH, length of the aquatic food chain, dissolved organic carbon, and temperature (EPA 2010).

C.4. Methylmercury Toxicology

C.4.1. Epidemiological Facts

The classical example of Hg poisoning occurred in Minamata bay in Japan. From this incident, a significant part of the knowledge on MeHg poisoning was obtain by study of victims; originally referred as "Minamata disease" (Laws 2018). More precisely, a neurotoxic poisoning originated by daily ingestion of large amounts of fish highly contaminated with MeHg. Large amounts of Hg^{2+} were discharged from a chemical factory in the Bay, then Hg^{2+} was converted to MeHg that polluted fish and shellfish (Ceccatelli 2012). About 100,000 people reported fish consumption in average between 286g of fish in the winter and 410g in the summer, daily per person (Laws 2018).

C.4.2. Neurotoxicity of Methylmercury

MeHg is primarily neurotoxic to adults and children. However, the fetus' developing brain due to rapid physiological changes and developmental protective system is highly vulnerable. Fetus' exposure to MeHg target the formation of key brain structures; altering brain's cortex, resulting in disruptive behavioral patterns. (Ceccatelli 2012). In Fetus, most of the functions of the CNS are formed during the third trimester of development, at this point brain appears to be vulnerable due to the transplacental transfer of neurotoxic chemicals including MeHg. The irreversible neurotoxic effects might not be detected at birth or first postnatal months of development but, they are noticed as the babies grow (Ceccatelli 2012).

Toxic effects of MeHg in adults include: sensory disturbance of the lower legs, lower arms, face, visual field constriction ("tunnel vision"), deafness, ataxia, and dysarthria (Ceccatelli 2012). In addition, neurological disturbance of intelligence, mood, behavior, diminished alterations on psychomotor functions (tremors in young adults <40), attention disorders, learning, and memory manifested by increased concentrations of Hg in hair (Ceccatelli 2012).

C.4.3. Methylmercury toxicity and Inhibition of Selenoenzymes

The toxicity of MeHg comprises a wide latency of onset of symptoms. Major pathological effects comprise cell's oxidative damage in affected tissues. Accentuated fetal vulnerability may result from low dietary Se (N. &. Ralston 2016). Se, is part of Selenocysteine, this amino acid is central to ~25 genetically unique selenoproteins present in humans. Some selenoproteins are vital enzymes that maintain intracellular homeostasis and brain conditions including prevention and reversal of reactive oxygen species, and free radicals (MeHg) effects; which promote oxidative damage (N. &. Ralston 2016). High MeHg concentrations follow these toxic paths:

C.4.3.1. Synergies of Sequestration

MeHg's binding of active sites of selenoenzymes which are important for catalysis of reactions. For instance, glutathione's function is to bring the thiol of sulfhydryls (-SH) to complete biochemical reactions but, because of MeHg's affinity for thiols, MeHg sequester the substrate of selenoenzymes (N. &. Ralston 2016).

C.4.3.2. Silencing of Selenoenzymes

Once MeHg sequester selenoenzymes' substrates, the presence of MeHg into the selenoenzyme active site form a MeHg-Selenocysteine inhibitor-enzyme inactive complex (N. &. Ralston 2016).

C.4.3.3. Sequestration of Selenium

MeHg's affinity for Se is 10^6 times bigger than those of analogous sulfur molecules (N. &. Ralston 2016). Consequently, high concentrations of MeHg are accumulated as mercury selenide (HgSe) in brain tissues and apparently arises as the breakdown product of MeHg-Selenocysteine in lysosomes (Korbas et al. 2010).

C.4.3.4. Suicide of Selenium-Deprived Cells

When cells cannot longer synthesize selenocysteine (Sec), because of MeHg binding. Then, they are called selenium-deprived cells. Sec is required for production of enzymes, but instead of them, production of truncated molecules promotes apoptosis (Anestål 2003).

APPENDIX D. SELENIUM LITERATURE REVIEW

D.1. Se General Facts

Selenium (Se), is a nonmetallic element found at trace levels in the human body. However, in large amounts have toxic properties. Berzelius (1779–1848) discovered several basic elements, including Se. (Hatfield 2012). Se, helps to protect intracellular components against oxidative damage (Medical Subject Headings 2017). Seleno-compounds such as selenoproteins: glutathione peroxidase and thioredoxin reductase are enzymes in charge of detoxification. They can be found alone or in combination with vitamin E acting as antioxidants (NCIt National Cancer Institute 2017).

Table D.1. General Properties of Se

Atomic number/structure	Se^{2+} 34
Isotopes	^{80}Se (most common), $^{74\text{-}82}Se$
Atomic weight	78.971 g/mol
Melting point	392^0F or 217^0C
Boiling point	685 ^0C or 1265 ^0F at 760 mm Hg
Density	4.28 g/cm^3
Viscosity	221 mPa-S at 220 ^0C
Vapor pressure	0.1 pascals at $20^0C \approx 0$ mmHg
Aqueous solubility	Insoluble

Source: (National Center for Biotechnology Information 2004).

Se's description: reddish colored powder but, may become black upon air exposure. It is also described in several forms of solid gray, amorphous or crystalline. Insoluble in water and alcohol, soluble in concentrated nitric acid, carbon disulfide, and ether (National Center for Biotechnology Information 2004).

D.2. Uses and Applications of Se

Se, is classified as inorganic substance with several applications summarized in table 5.2

Table D.2. Applications of Se

Electronics industry
Glass industry
Pigments in plastics, paints & inks
Vulcanizing agent in rubber industry
Catalyst for Pharmaceuticals
Cosmetic shampoo
Fungicides, pesticides, & agriculture-chemicals
Food industry, nutritional additive.

Source: (National Center for Biotechnology Information 2004)

Therapeutic uses of Se are found in experimental therapy. The study of seleno-methyl-selenocysteine; considered one of the most effective chemo-preventive Se compounds. Furthermore, at Nano scale, Se can increment the activities of glutathione peroxidase and other selenoenzymes with lower toxicity risk (National Center for Biotechnology Information 2004). Furthermore, Se is a trace element for the human body it is present in 25-35 seleno-enzymes with vital functions for the brain and endocrine system (Ralston 2016).

D.3. Toxicological Facts

Se, is not classifiable as human carcinogenic (HSDB 2017). Common effects on health include: irritation of eyes, nose, throat, and skin with moderate effect, cough, visual disturbance, headache, fever, weakness, dyspnea, bronchial spasms, bronchitis, pulmonary edema, metallic taste, garlic breath, GI disturbance, tachycardia, and tremors. Exposure routes are absorption by inhalation, ingestion and contact with eyes or skin. It has cumulative systemic toxicity by chronic exposure: discoloration of skin, thickened and brittle nails; nail and hair loss, excessive tooth decay (yellowish), lack of mental alertness; mood changes (depression, irritability) (OSHA 2017).

D.4. Environmental Se

Se, is abundantly distributed mainly from volcanic origin. It occurs as inorganic oxides: selenate and selenite, as elemental Se, and selenide or combined with metals, as in ferroselite, coal and oil deposits (Coyne 2013). Se, is considered very toxic to aquatic organisms (ILO-ICSC 2017). Speciation of Se is influenced by redox potential and pH in water; low pH and reducing conditions favor elemental Se (HSDB 2017). In sediments, reduced and tightly bound Se stay immobile unless the sediments are chemically or biologically oxidized (HSDB 2017).

D.5. Role of Selenoproteins in Humans

Most of Se found in biological systems is present as selenocysteine (Sec). Therefore, Se's role in biology is because of its occurrence in proteins (enzymes) in the form of Sec. For example: Selenoproteins utilize Sec in redox catalysis (Hatfield et al. 2012).

D.5.1. Selenoproteins in mammals: Glutathion Peroxidases

There are up to eight glutathione peroxidases in mammals; five are Sec containing enzymes (GPx1, GPx2, GPx3, GPx4, and GPx6) (Hatfield 2012). GPx1 is the most abundant in mammals, catalyzes glutathione-dependent hydroperoxide reduction (Hatfield 2012).

D.5.2. Thyroid Hormone Deiodinases and Other Families of Selenoenzymes

Three deiodinases are found in mammals: DI1, DI2 and DI3. They activate or inactivate thyroid hormones through reductive deiodination. Deiodinases are thioredoxin-fold proteins (Hatfield 2012). The family of thioredoxin reductases comprises TR1, TR2, and TR3.All essential for cellular and embryonic processes. (Hatfield 2012). Other important selenoproteins are Methionine- R -Sulfoxide Reductases, kDa, Selenophosphate Synthetase 2, and Selenoproteins T, M, H, K, N, S, P, W, and O. For a complete description of their function refer to (Hatfield 2012).

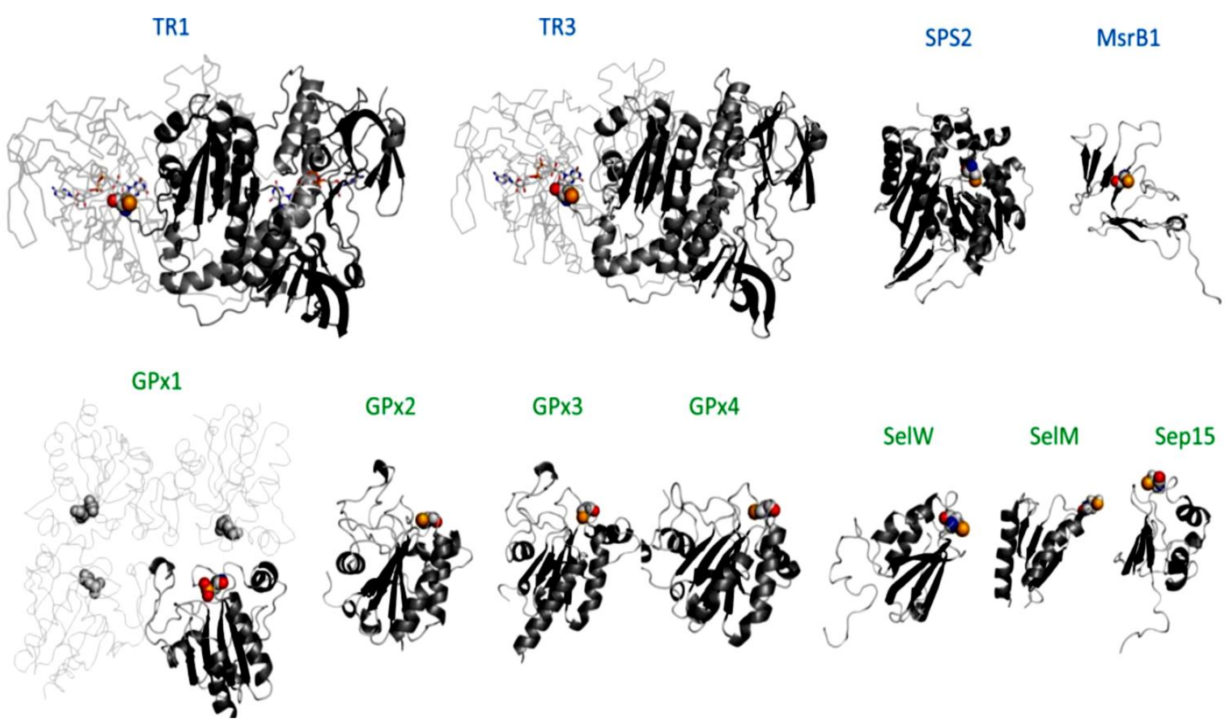

Figure D.1. Tridimensional structures Selenoproteins (Hatfield 2012).

D.5.3. General Functions of Selenoproteins

Apparently, the general function, of most selenoproteins are oxidoreductases. In the structure of these proteins, Sec is the active catalytic residue used, resulting in the reversible change of Sec's redox state during catalysis (Hatfield 2012). Many Se-containing amino acids found in animal proteins are similar to sulphur-containing amino acids. For instance, methionine and seleno-methionine in image 6 (Gates 2016).

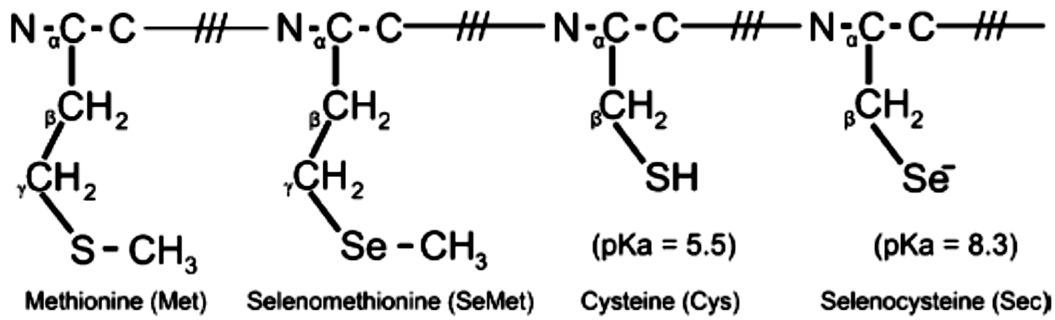

Figure D.2. Sulphur and Se-containing amino acid (Gates 2016).

D.6. Seafood Safety and the Benefits of Dietary Selenium

Ocean fish is rich in essential nutrients required for normal physiological functions. Fish's nutritional sources of low-fat protein, omega-3 polyunsaturated fatty acids, vitamins A and D, iodine, Se, and other micronutrients (Gates 2016). Thus, consumption of fish during pregnancy is markedly beneficial. As discussed previously, Se is essential in diet; many metabolic processes, several diseases and clinical issues are related to disruptions on selenoenzymes. One American rich source of dietary Se, is ocean fish (Gates 2016). Moreover, fish revealed higher Sec content and Se in tissue, compared to mammals (Hatfield 2012). While their fillets concentrations vary significantly by species; it typically remains fairly constant for all type of fish despite of its size. Several organisms in nature use Sec to protect their brain tissue from oxidative damage which results of normal cellular respiration. Ocean fisheries hold around 30-37 Sec containing proteins (Gates 2016). Moreover, other varieties of nonprotein molecular forms of Se can be found in ocean fish. Se in fish happens to be available for Sec synthesis (Ralston and Raymond 2010).

D.7. Se Vs. Hg: Protection Against MeHg Toxicity

The toxic MeHg effects might depend on dietary Se consumption. A low dietary Se intake might increase the risk of MeHg neurotoxicity (Ralston 2010).

D.7.1. Se the nutraceutical

A nutraceutical is food that have medical or health benefits related to prevention or treatment of disease. Se, is a vital trace element with important benefits to humans: growth factor, powerful antioxidant, anticancer properties, and role on normal thyroid hormone homeostasis and immunity (Ralston et al. 2010).

D.7.2. The binding affinity argument

Preliminary ideas suggested that dietary Se incorporated in the diet may bind to MeHg preventing Hg toxicity. The Se's affinity for Hg, binding together produce insoluble mercury selenides (HgSe) that is retain in the brain, but they are metabolically inert (Ralston 2010). Hg's affinity for the sulfur of cysteine is 10^{14}, but Hg's affinity for the Se of Sec is estimated to be $\sim 10^{22}$. The selenides high affinity constant for Hg (10^{45}) is a million times higher than that of sulfide (10^{39}), mercury's second-best binding partner (Ralston 2010).

D.7.3. Se from molecular target to tonic

This argument reverses the previous explanation. It is Hg's propensity for Se sequestration occurring in brain or endocrine tissues what may inhibit the production of selenoproteins, depending on dietary Se levels. Hence, supplemental Se may exert the protective effect if it's present in acceptable levels to keep Se available for substitution of Se lost by MeHg sequestration, keeping normal selenoprotein synthesis (Ralston 2010).

D.8. The Hg-Se Fish's Molar Ratio

MeHg accumulation is uncontrolled but, Se in tissues is homeostatic regulated. Thus, molar ratios of Se:Hg in seafood are prone to variations directly related to MeHg (Hatfield 2012). It is expected to find more Se than Hg in seafood. Nonetheless, some species of shark and pilot whale are exceptions containing Hg in molar excess (Hatfield 2012).

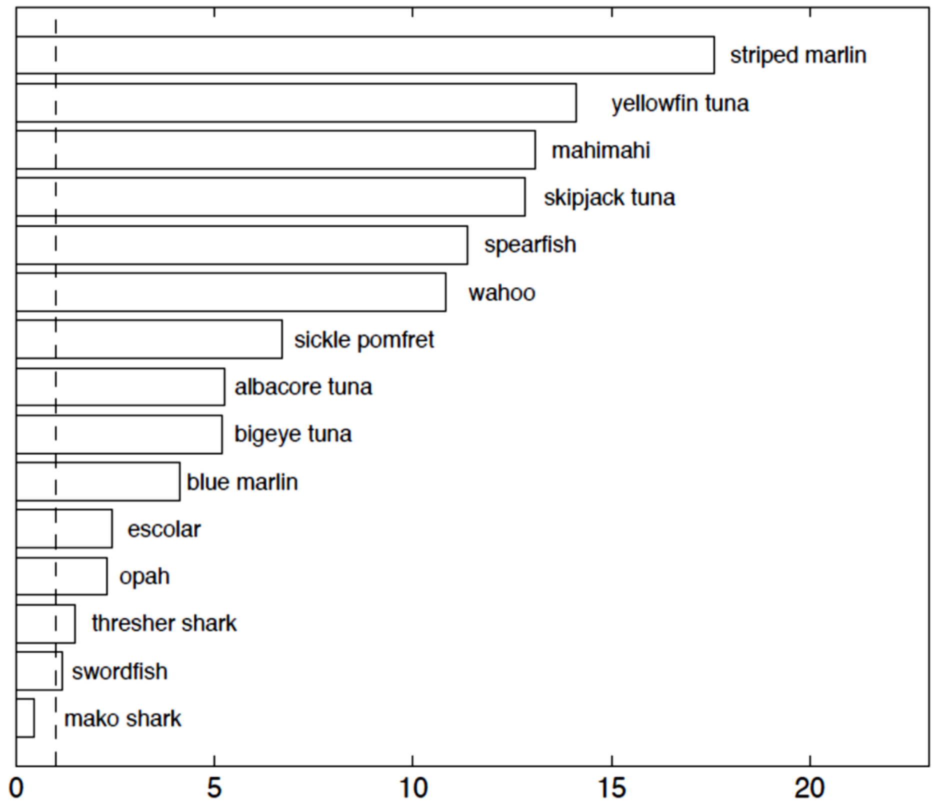

Figure D.3. Molar ratios Se:Hg in marine species (Laws 2018)

Furthermore, maternal dietary Se:Hg ratios need to be significantly lower than 1.0 to keep maternal supply of Se to the fetus and preventing loss of selenoenzyme functions (Hatfield 2012). When MeHg exceed or approach 1:1 molar ratio to Se, it would induce also toxicity secondary to selenoenzyme inhibition by exchange binding partners (sulfur or other cellular structures) (Ralston 2010). The most important commercial ocean fish species tend to keep molar ratios Se:Hg quite low for fish muscle, this comprises 17 of 25 top sources of Se in the American diet (Kaneko 2007). The ratio Se:Hg is now an important risk criterion to evaluate exposure to Hg instead of Hg alone (Kaneko & Ralston 2007).

D.9. The Se Health Benefit Value

The description of Se's nutritional benefits related to potential risk of MeHg exposure. the Se health benefit value was proposed by Kaneko and Ralston (2007):

$$\text{Se HBV} = (\text{Se/Hg molar ratio x total Se}) - (\text{Hg/Se molar ratio} \times \text{total Hg})$$

D.10. Ocean Fish Vs. Freshwater Fish

Ocean fish is rich in Se, but in freshwater fish the risk of MeHg exposure may be diverse because regional and particular differences in Se intake. Factors affecting Se intake include: variability of Se availability for any environment, Se abundance in soils of specific areas or the risk of low regions only a few miles away, and geological distributions of Se. The content of Se for freshwater fish is more variable and might be low in some regions. Even worse, fish from low-Se lakes tend to have higher MeHg content, a dangerous combination for pregnant women (Ralston 2010).

APPENDIX E. METHYLMERCURY SCENARIO IN LOUISIANA AND ADVISORIES

E.1. The mercury issue in Louisiana

Monitoring Hg in Louisiana is responsibility of the Department of Environmental Quality of Louisiana (LDEQ) in collaboration with other state agencies: Louisiana Department of Health and Hospitals (LDHH) and the Louisiana Department of Wildlife and Fisheries (LDWF). Their purpose is to assign skilled people to hunt Hg in waterbodies where Hg might be a problem. Then, provide information to the public, so they can make informed decisions to reduce their risk of exposure (LDEQ 2003).

Louisiana is known as the “sportsman’s paradise” due to the rich natural resources; then, fishing and hunting are very popular activities (LDEQ 2003). Eating fish is a healthy habit but, unfortunately, certain fish coming from Louisiana’s water bodies may contain MeHg. The objective of LDEQ is to reduce the risks associated to Hg exposure (LDEQ 2003).

E.2. Reducing Risk

The most powerful tool promoted by LDEQ is being informed, read, and understand the recommendations for eating fish and all advisories concerning Hg. The advisories’ goal is avoiding consumption of larger amounts of certain fish species or intake of predatory species such as: largemouth bass, bowfin, king mackerel, and shark (LDEQ 2003).

Furthermore, LDEQ recommends having a diet based on a variety of fish coming from various water bodies. This may help to reduce the exposure to “hot spot” species and areas. LDEQ is in charge to post visible advisory signs, near waterbodies under the advisory status. They contain information including: the contaminants responsible for the advisory, types of fish affected, how much fish can be ingested safely, and the area range covered by the advisory. (LDEQ 2003).

Figure E.1. LDEQ advisory (LDEQ 2003)

Several offices comprise the Hg program division of the LDEQ. One of the most important is the surveillance division. Some of their functions encompasses sampling of fish, water, sediments and, some plants for analysis of Hg. Waterbodies with Hg advisories are re-sampled annually, depending on State's budget. The personnel are assigned to go to selected waterbodies around the state for sample collection. Under Louisiana's legislature the need for fish collection, laboratory analysis, posting advisory signs and dissemination of information to the public, is recognized, and funded since 1993 (LDEQ 2003).

E.3. Fish species present in Louisiana's Advisories

The most frequently reported fish species in Louisiana's Hg advisories comes from 29 freshwater advisories where bowfin and Largemouth bass are the most frequent (LDEQ 2003).

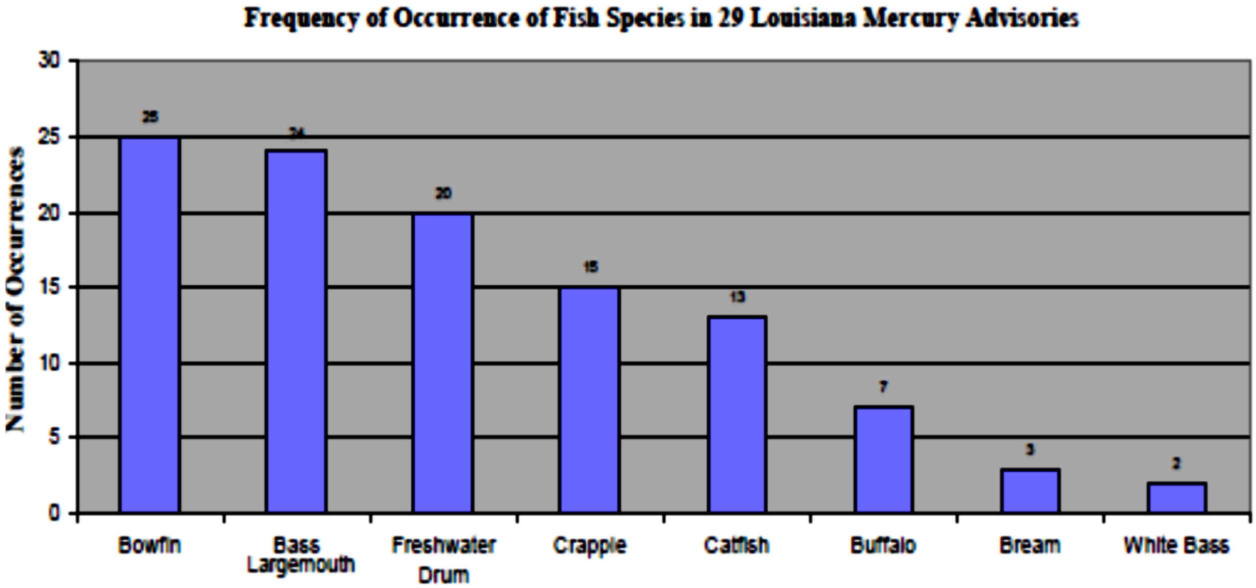

Figure E.2. Fish under LA advisories (LDEQ 2003)

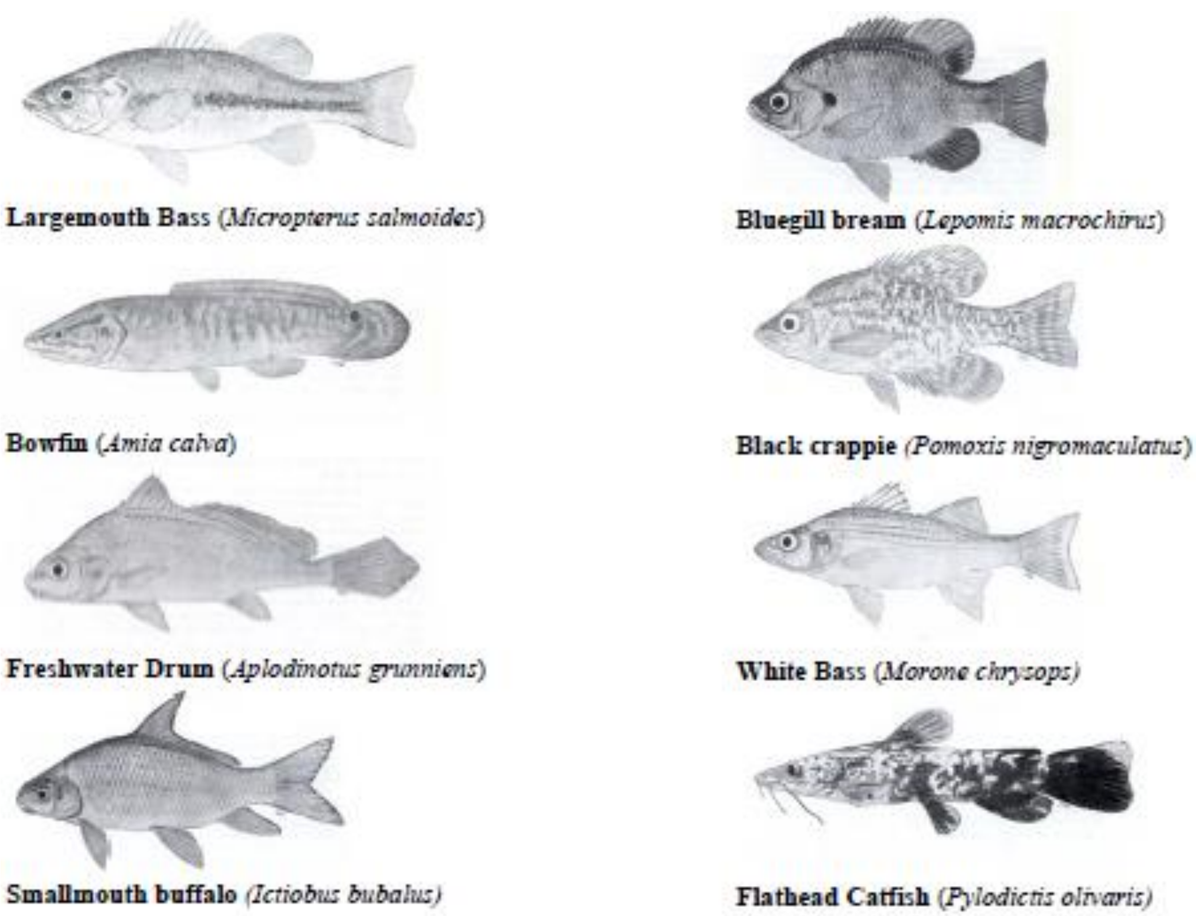

Figure E.3. Common fish in LA advisories (LDEQ 2003).

E.4. Mercury Advisories in Louisiana

The advisories in Louisiana use an approach of “limited-meal” within a specific area. The posted advisory contains this legend: “Unless the fish species is specifically addressed in the details of the advisory, please limit consumption of all species in an advisory area to 4 meals of fish per month. Louisiana fish consumption advisories are based on the estimate that the average Louisiana resident eats no more than 4 meals of fish per month (1 meal = ½ pound).” In some cases, there is an extra advise for woman in childbearing age, other adults, and children. (LDEQ 2003).

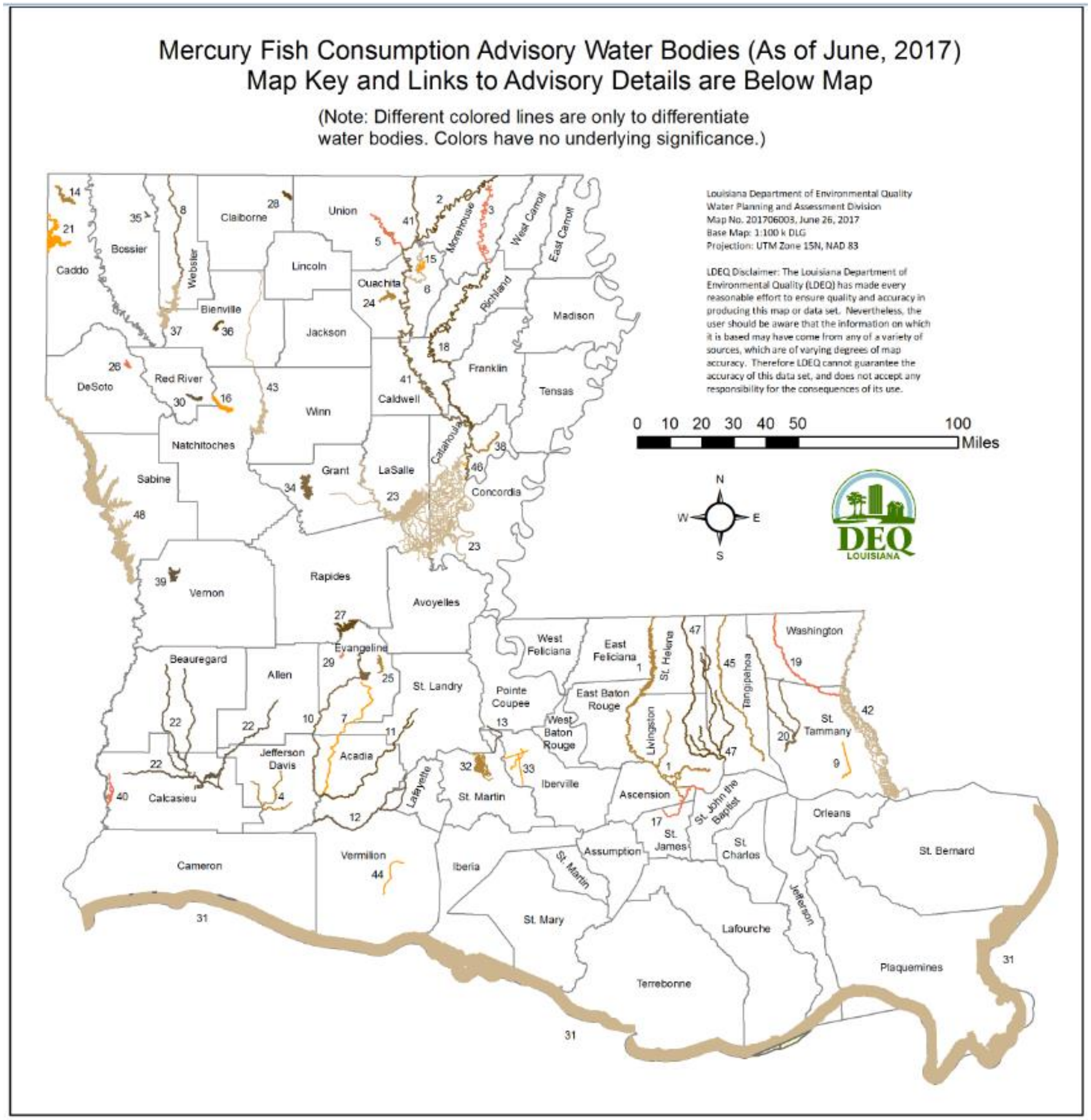

Figure E.4. Updated map of LA Advisories (LDEQ February 9, 2018)

Mercury Advisory Map Key and Links to Advisory Details			
Map Number	Water Body Name	Map Number	Water Body Name
1	Amite River Drainage Basin	25	Chicot Lake
2	Bayou Bartholomew	26	Clear Lake (Lake Edwards)
3	Bayou Bonne Idee	27	Cocodrie Lake
4	Bayou Chene and Bayou Lacassine	28	Corney Lake
5	Bayou De L'outre and Associated Lakes	29	Crooked Creek Reservoir
6	Bayou De Siard	30	Grand Bayou Reservoir
7	Bayou des Cannes	31	Gulf of Mexico off Louisiana Coast
8	Bayou Dorcheat	32	Henderson Lake area including Lake Bigeux
9	Bayou Liberty	33	I-10 Canal and Work Canal and Bayou Bristow
10	Bayou Nezpique	34	Iatt Lake
11	Bayou Plaquemine Brule	35	Ivan Lake
12	Bayou Queue De Tortue	36	Kepler Creek Lake
13	Big Alabama Bayou	37	Lake Bistineau
14	Black Bayou Lake (Caddo Parish)	38	Lake Louis (Lovelace Lake) and Bayou Louis
15	Black Bayou Lake (Ouachita Parish)	39	Lake Vernon
16	Black Lake	40	Old River (Niblett Bluff)
17	Blind River	41	Ouachita River
18	Boeuf River	42	Pearl River
19	Bogue Chitto River	43	Saline Bayou and Saline Lake
20	Bogue Falaya and Tchefuncte Rivers	44	Seventh Ward Canal
21	Caddo Lake	45	Tangipahoa River
22	Calcasieu River Drainage Basin	46	Tew Lake
23	Catahoula Lake, Little River, Old River, Black River, Saline Lake, Larto Lake (Saline/Larto Complex), Shad Lake & Associated Water Bodies	47	Tickfaw River Drainage Basin
24	Cheniere (Brake) Lake	48	Toledo Bend Reservoir

Figure E.5. Updated waterbodies under advisory (LDEQ February 9, 2018)

The federal government agencies collaborating in issuing advisories include the U.S. EPA, and the FDA. Hg and its species are listed as toxic pollutants under section 307(a) of the Clean Water Act (see 40 CFR 401.15). EPA's Hg advisories in most cases is 1 ppm. The National Listing of Fish advisories defined the total number of statewide advisories by 2011:

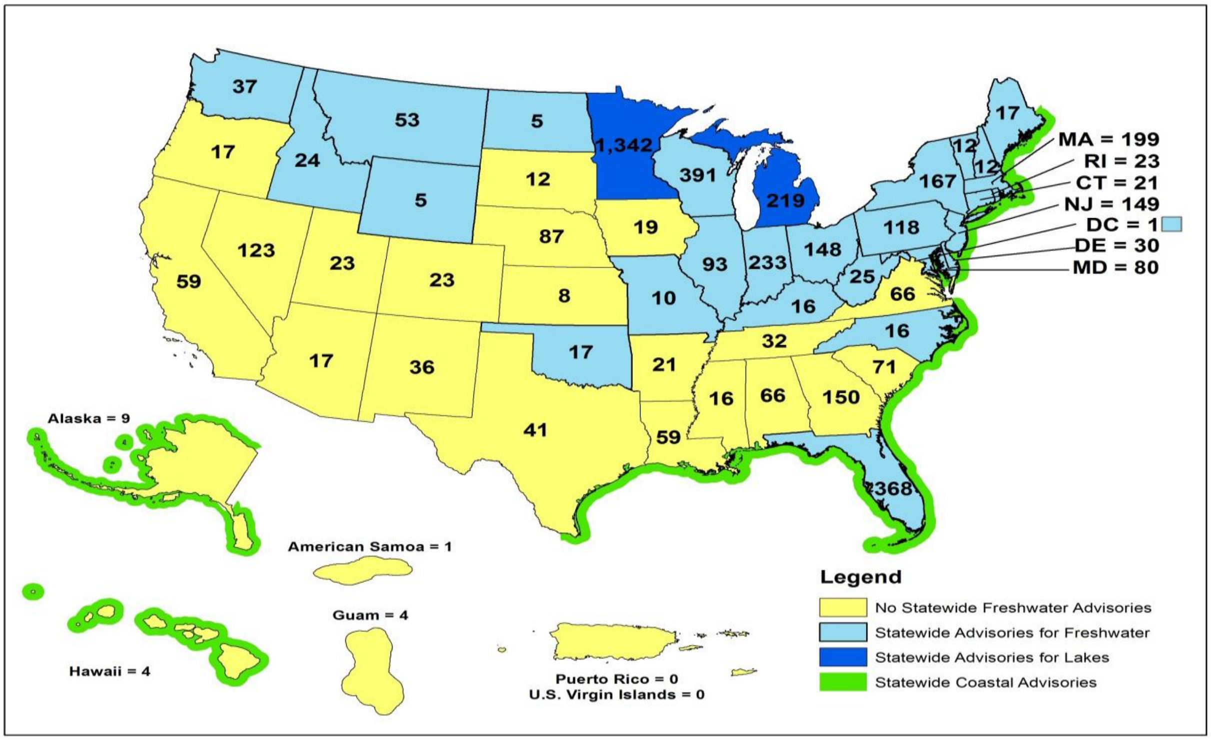

Figure E.6. Statewide advisories. Source: 2011 National Listing of Fish Advisories.

E.5. FDA & EPA Advisories

In 2017, both federal agencies issued an advice about eating fish and shellfish: They suggested that childbearing age women (16-49 years old), pregnant and breastfeeding women, and young children are groups of people that should eat more fish that is lower in Hg for health care (EPA 2017).

The Advisory stated: "women and children should eat 2-3 servings (8-12 ounces for adults and children over age 10, smaller amounts for younger children) of a variety of fish and shellfish each week. The advice includes a chart showing how often to eat more than 60 types of fish and shellfish and supplemental questions and answers." (EPA 2017).

E.6. Basis for Issuing Public Health Advisories in Louisiana

The authorities in charge of designing the protocol for fish and shellfish advisories are: The Louisiana Department of Health and Hospitals (LDHH) in coordination with the Louisiana Department of Environmental Quality (LDEQ), Louisiana Department of Wildlife and Fisheries (LDWF) and Louisiana Department of Agriculture and Forestry (LDAF). The developing of the protocol for issuance of advisories usually follow these steps: research to find a pollutant in fish tissue, analysis to determine the need for an advisory, and the ultimate interagency consultation. Advisories are specific for each waterbody. See diagram 1 (LDHH 2012).

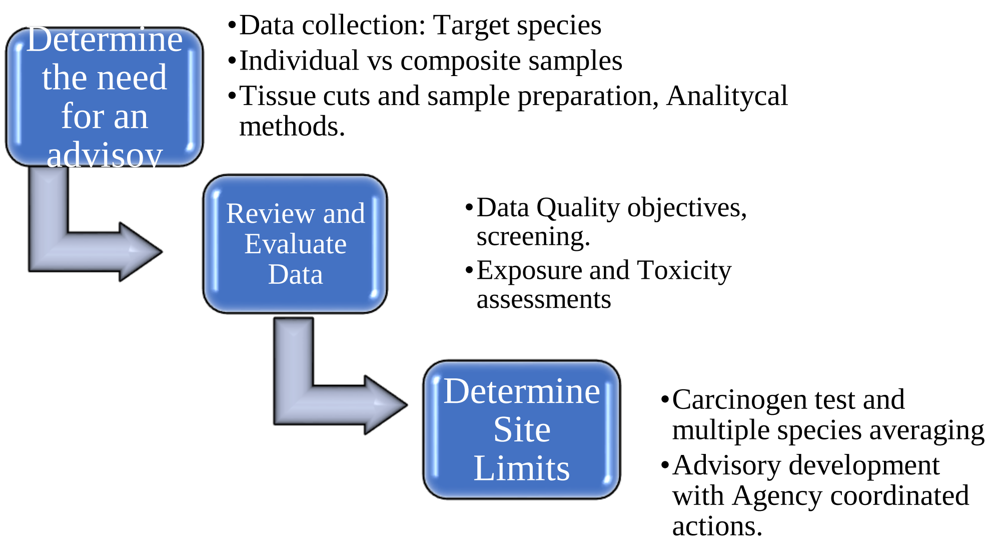

Figure E.7. Developing advisories (LDHH 2012).

the re-evaluation of the advisory is based on the newly calculated annual average of the pollutant in fish tissue concentrations. if the arithmetic mean of concentrations in shellfish or fish for every single sampling event were acceptable, and for at least three consecutive sampling events for a period of two years at least, then, the advisory might be rescinded (LDHH 2012).

E.7. Recreational Anglers in Louisiana

Recreational anglers might be highly exposed due to their high ingestion of wild-caught fish. They may exhibit high MeHg concentrations (Lincoln et al.2011). According to the U.S. Fish and Wildlife Service, in 2006, around 780,00 Louisiana residents purchased a recreational fishing license (2009); including anglers and nonanglers, which also reported high consumption of fish (Lincoln et al. 2011). In a study conducted by Lincoln et al., in 2006, they surveyed 534 anglers. Analytical measurements of total Hg were made from hair samples from 402 surveyed anglers. Anglers' median hair Hg concentration was 0.81 μg/g; 40% of participants had levels >1 μg/g, which corresponds to the EPA's reference dose (Lincoln et al. 2011).

APPENDIX F. METHYL MERCURY ANALYSIS

Table f.1: Results of Methylmercury analyses of 57 samples (including duplicates) of fish via Direct Mercury Analyzer.

Table F.1. Methylmercury Analysis d*= duplicate, w/w= wet weight filet.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	$HgCH_3$ (nanograms)	$HgCH_3$ (ppm)
Black Drum	Calcasieu Lake	S1	185.94	n/a	0.1004	3.1732	**0.032**
Black Drum	Calcasieu Lake	S1d	185.92	n/a	0.1159	2.7293	**0.024**
Black Drum	Calcasieu Lake	S2	113.78	n/a	0.1115	3.6173	**0.032**
Black Drum	Calcasieu Lake	S3	125.58	n/a	0.1092	3.6314	**0.033**
Black Drum	Calcasieu Lake	S4	130.05	n/a	0.1169	2.6865	**0.023**
Black Drum	Calcasieu Lake	S5	150.34	n/a	0.1055	2.8312	**0.027**
Black Drum	Calcasieu Lake	S5d	150.37	n/a	0.1113	4.3553	**0.039**
Black Drum	Calcasieu Lake	S6	178.42	n/a	0.0951	0.9027	**0.009**
Black Drum	Calcasieu Lake	S7	105.73	n/a	0.1002	4.0006	**0.040**
Black Drum	Calcasieu Lake	S8	93.99	n/a	0.1103	2.4553	**0.022**
Black Drum	Calcasieu Lake	S9	110.80	n/a	0.1035	0.9814	**0.009**
Black Drum	Calcasieu Lake	S10	90.97	n/a	0.1115	2.4136	**0.022**
Black Drum	Calcasieu Lake	S10d	90.93	n/a	0.1036	2.6823	**0.026**
Cat Fish	Toledo Bend	S1	84.71	n/a	0.1077	1.2451	**0.012**
Cat Fish	Toledo Bend	S1d	84.71	n/a	0.1102	0.7878	**0.007**
Cat Fish	Toledo Bend	S2	134.24	n/a	0.1143	1.164	**0.010**
Cat Fish	Toledo Bend	S3	120.31	n/a	0.0998	0.9202	**0.009**
Cat Fish	Toledo Bend	S4	108.28	n/a	0.1035	0.6767	**0.006**
Cat Fish	Toledo Bend	S5	91.57	n/a	0.1079	0.9412	**0.008**
Cat Fish	Toledo Bend	S6	113.09	n/a	0.0955	1.1675	**0.012**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	$HgCH_3$ (nanograms)	$HgCH_3$ (ppm)
Cat Fish	Toledo Bend	S7	186.99	n/a	0.1067	2.5629	**0.024**
Cat Fish	Toledo Bend	S8	131.88	n/a	0.1117	0.619	**0.006**
Cat Fish	Toledo Bend	S9	96.71	n/a	0.0976	1.0194	**0.010**
Cat Fish	Toledo Bend	S10	156.50	n/a	0.1046	0.642	**0.006**
Cat Fish	Toledo Bend	S10d	156.60	n/a	0.1064	0.7084	**0.006**
Large Mouth Bass	Atchafalaya	S1	157.33	33.66	0.0928	0.4047	**0.004**
Large Mouth Bass	Atchafalaya	S1d	157.33	34.29	0.0962	0.4253	**0.004**
Large Mouth Bass	Atchafalaya	S2	163.36	30.48	0.0956	0.7183	**0.007**
Large Mouth Bass	Atchafalaya	S3	141.70	34.29	0.115	0.3767	**0.003**
Large Mouth Bass	Atchafalaya	S4	216.89	35.56	0.1047	0.5039	**0.005**
Large Mouth Bass	Atchafalaya	S5	171.16	27.94	0.1123	0.6697	**0.006**
Large Mouth Bass	Atchafalaya	S5d	171.16	30.48	0.1153	0.7773	**0.007**
Large Mouth Bass	Atchafalaya	S6	117.18	38.1	0.0982	0.4729	**0.005**
Large Mouth Bass	Atchafalaya	S7	135.36	31.75	0.11	0.5729	**0.005**
Large Mouth Bass	Atchafalaya	S8	277.33	31.75	0.0986	0.8888	**0.009**
Large Mouth Bass	Atchafalaya	S9	148.55	31.75	0.0994	0.3801	**0.004**
Large Mouth Bass	Atchafalaya	S10	143.94	12.7	0.0988	0.4488	**0.004**
Large Mouth Bass	Atchafalaya	S10d	143.94	15.88	0.1223	0.5774	**0.005**
Blue Gill	University Lake LSU	S1	41.27	20.32	0.101	0.7994	**0.008**
Blue Gill	University Lake LSU	S2	69.79	19.05	0.1065	1.1816	**0.011**
Large Mouth Bass	University Lake LSU	S3	118.70	12.06	0.1043	1.3065	**0.013**
Large Mouth Bass	University Lake LSU	S4	83.60	16.51	0.1135	1.7709	**0.016**
Blue Gill	University Lake LSU	S5	31.28	25.4	0.1133	0.5815	**0.005**
Blue Gill	University	S5d	31.28	12.7	0.1046	0.5798	**0.006**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	$HgCH_3$ (nanograms)	$HgCH_3$ (ppm)
	Lake LSU						
Blue Gill	University Lake LSU	S6	105.16	15.24	0.1063	0.5005	**0.005**
Brown Bowl Head Catfish	University Lake LSU	S7	344.58	13.97	0.1026	1.3151	**0.013**
Blue Gill	University Lake LSU	S8	38.29	13.97	0.1154	0.9495	**0.008**
Blue Gill	University Lake LSU	S9	68.30	15.88	0.1071	0.5022	**0.005**
Blue Gill	University Lake LSU	S10	59.56	17.78	0.1035	0.5367	**0.005**
Blue Gill	University Lake LSU	S10d	59.56	13.97	0.0992	0.5034	**0.005**
Blue Gill	University Lake LSU	S11	49.42	13.33	0.1044	0.8143	**0.008**
Large Mouth Bass	University Lake LSU	S12	51.53	15.87	0.1137	0.8091	**0.007**
Large Mouth Bass	University Lake LSU	S13	74.78	17.78	0.1029	1.2151	**0.012**
Blue Gill	University Lake LSU	S14	43.82	17.78	0.103	0.8022	**0.008**
Blue Gill	University Lake LSU	S15	45.70	45.72	0.1263	0.8034	**0.006**
Blue Gill	University Lake LSU	S15d	45.70	38.1	0.1071	0.79	**0.007**
Gizzard Shad	University Lake LSU	S16	61.72	30.48	0.1091	0.0666	**0.001**
Gizzard Shad	University Lake LSU	S17	59.11	30.48	0.106	0.0984	**0.001**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S1	101.27	33.02	0.1159	6.5407	**0.056**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S1d	101.27	30.48	0.1062	5.7313	**0.054**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S2	51.05	35.56	0.118	6.1769	**0.052**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S3	30.80	27.94	0.1042	4.3237	**0.042**
Large	Henderson	S4	18.44	27.94	0.1113	5.6155	**0.051**

table cont'd.

Species	Location	Sample #	Total Weight (w/w, g)	Length (cm)	Sample (g)	$HgCH_3$ (nanograms)	$HgCH_3$ (ppm)
Mouth Bass	Lake, Breaux Bridge						
Large Mouth Bass	Henderson Lake, Breaux Bridge	S5	25.38	33.02	0.1152	5.9491	**0.052**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S5d	25.38	33.02	0.1025	5.1187	**0.050**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S6	20.08	33.66	0.1071	5.3669	**0.050**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S7	25.42	34.29	0.1168	6.5213	**0.056**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S8	16.12	30.48	0.1112	3.8554	**0.035**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S9	17.29	34.29	0.104	4.4813	**0.043**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S10	21.03	35.56	0.1021	4.0867	**0.04**
Large Mouth Bass	Henderson Lake, Breaux Bridge	S10d	21.03	27.94	0.1112	4.4004	**0.040**

VITA

Alexander Reyes-Avila, born in Tegucigalpa, Honduras. Alexander worked for the faculty of Chemistry and Pharmacy of Honduras prior to earn his bachelor’s degree in Chemistry from the National University of Honduras. Two years later he pursued a MBA in the Technological Centro American University, and eventually, began to work as full-time Research Assistant Professor for the National University of Honduras. There, he participated in projects related to water quality issues and conducted research at the university campus to assess water quality supplied to the campus population. As his interest in environmental issues and water quality grew, he got admission to the Department of Environmental Sciences at Louisiana State University in 2016. Upon accomplishment of his master’s degree on August 2018, he will look for an opportunity to get a Doctorate, in order to go deep in water pollution issues.